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APPLICATIONS OF RECENT ADVANCES IN NUCLEAR PHYSICS TO CANCER RESEARCH.

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THE immediate influence of the recent developments in nuclear physics upon the study of cancer is likely to be due to the provision of new and powerful tools for investigation and of new agents with therapeutic possibilities. It is more difficult to assess the significance of the studies of the mechanism of the biological action of radiations necessitated by the protection of large numbers of industrial workers from the radio-active hazards involved in making atomic bombs (R. S. Stone, 1946).

These subjects have been discussed in two previous papers (Mitchell, 1946, *a*, *b*). The present review includes more recent published information, and emphasizes different aspects.

The most important technical development is, of course, the chain-reacting "pile" (Smyth, 1945). This provides a method of preparation of very large amounts of many radio-active isotopes. It seems likely that these substances will ultimately become widely applied in medicine and biology as:

1. Tracers for the study of normal and pathological metabolic processes and in investigations in pharmacology and therapeutics.
2. Artificial radio-active sources mainly for use in radiotherapy as a substitute for radium.
3. Agents with therapeutic possibilities such as radio-phosphorus and the radio-iodines, and probably much better, suitable organic compounds containing radio-active isotopes, whose action depends on selective concentration of the radio-activity in particular cells.

The separation on the industrial scale of non-radio-active isotopes and the practical development of the mass spectrometer open new possibilities of isotopic tracer research where radio-active effects are undesirable, as in many clinical investigations, or where, as in the case of nitrogen and oxygen, no suitable radio-active isotopes exist.

The cyclotron still remains an essential instrument both for the production of small amounts of certain isotopes and as a source of fast neutrons for therapeutic trials in cancer and related diseases. It is by no means certain that the frequency modulated cyclotron will be useful as a therapeutic source of fast

neutrons, on account of the possibility of reduced neutron output and of the undesirability of a pulsed output.

A promising field for radiotherapeutic investigation appears to be the study of high energy, 20–50 Mev. gamma radiation. The newly developed synchrotron appears to provide a practical source of high energy, e.g. 30 Mev. gamma radiation, much more easily obtainable than the betatron. It is possible that the linear accelerator may prove more suitable than the synchrotron. Although the electron beam can now be extracted from these machines, the therapeutic possibilities of the high energy beta particles are rather uncertain and must be investigated with caution.

The development of high energy generators opens the interesting possibility of the radiotherapeutic application of fast protons of energy in the region of 140 Mev. (Wilson, 1946).

The results of selected investigations are best summarized in the discussion of these newer agents.

Properties and Production of Isotopes, Radio-active and Stable.

The properties of selected isotopes of especial interest are summarized in Table I. Recent information on isotopes, which may be useful as radio-active tracers, and which are produced in the pile, is summarized in Table II. A list of recent references on isotopes is also given.

The pile is without a rival for producing isotopes in the very large number of cases where, during irradiation in the pile, a simple slow neutron capture process occurs, e.g. $P^{31} + n \rightarrow P^{32}$, with emission of gamma radiation. Examples of specific activities obtainable by $n - \gamma$ reactions in the pile are for P^{32} one-third curie per g. and for S^{35} 10 millicuries per g.

There is one slow neutron (n — p) reaction of especial importance: $N^{14} + n \rightarrow C^{14} + p$. It is of interest that this process is probably responsible for 50–80 per cent of the biological effects of thermal neutrons, the remainder of the effects being due almost entirely to the $n - \gamma$ reaction in hydrogen (Mitchell, 1947). However, the n, p reaction in nitrogen is of extreme importance for isotopic tracer research as the basis for the preparation of C^{14}, the long-lived radio-active isotope of carbon. It has been shown by Yankwich, Rollefson and Norris (1946) that the C^{14} is produced by slow neutron irradiation of compounds such as ammonium nitrate and urea in the form of very simple compounds such as CO_2,CO,CH_3OH,H.COOH, and of especial importance as a starting-point for organic syntheses, HCN.

In addition to the preparation of materials by direct irradiation, a very considerable number of radio-active isotopes are found as fission products in the uranium rods in a pile. A most valuable detailed list of these has been given by the Plutonium Project (1946).

In general, for the preparation of isotopes the yields with the cyclotron are very much smaller than those with the pile. A list of isotopes of interest made with the cyclotron is given in Table IV. The cyclotron can be used to prepare small amounts of *any* isotope. Further, the cyclotron is an essential supplement to the pile in order to produce—

(*a*) Alternative isotopes not made by slow neutron reactions, e.g. Na^{22} (half-life 3·0 years), Fe^{55} (half-life approximately 4 years).

(*b*) Higher concentrations of activity where preparation in the cyclotron involves a change in element and hence chemical separation is possible, e.g. As^{74} (half-life 16 days).

(*c*) Short-lived isotopes, e.g. C^{11} (half-life 20·4 mins.), N^{13} (half-life 9·93 minutes), F^{18} (half-life 112 minutes) and I^{130} (half-life 12·6 hours), which is accompanied by smaller amounts of I^{131} (half-life 8·0 days).

The methods used in the separation of stable isotopes have been reviewed by Thode and Reid (1946) and briefly by Urey (1946). The hydrogen isotopes, H^1 and H^2, have been separated on a large scale by distillation of water and the exchange reaction between water and hydrogen gas. The carbon isotopes have been partially separated by thermal diffusion and more cheaply by two chemical exchange reactions. The nitrogen isotopes have been separated by the exchange reaction between the ammonium ion in solution and ammonia gas and are now available commercially in U.S.A. The oxygen isotopes have been concentrated by distillation of water and by exchange reactions. Sulphur has been separated by chemical exchange between SO_2 and the bisulphite ion. Potassium has been also separated by chemical exchange methods.

The development of the mass-spectrometer as a practical instrument and its routine use for hydrocarbon analyses by industrial laboratories in U.S.A. show clearly the possibility in medicine and biology of non-radio-active isotopic tracer investigations. From this point of view, although deuterium still has its uses, the stable isotopes of greatest interest are C^{13}, N^{15}, O^{18} and S^{34}.

A valuable little book entitled 'Preparation and Measurement of Isotopic Tracers' (edited by D. Wright Wilson (1946), J. W. Edwards, Ann Arbor), includes detailed accounts of the mass spectrometer and of the estimation of C^{14}, in addition to useful information about measurements with Geiger counters.

Isotopic tracers.

The first medical application of radio-active isotopic indicators appears to have been the investigation by Hevesy (Christiansen, Hevesy and Lomholt, 1924) of the absorption, distribution and excretion of bismuth in connection with the treatment of syphilis. Since then a surprisingly large literature of biochemical applications of stable and radio-active tracers has grown up. Interesting reviews have been published recently by Hevesy (1946) and Rittenberg and Shemin (1946).

Probably the most remarkable result of general biological importance emerging from tracer studies, mainly by Hevesy and Schoenheimer, is the discovery of the dynamic state of the tissue constituents. Almost all the constituent molecules of the animal body are continually broken down and resynthesized, so that the adult living cell is literally in a state of "dynamic equilibrium."

The most important application of tracers in radiotherapeutics so far has been the work of Hevesy (1945), who used radio-phosphorus (P^{32}) to demonstrate inhibition of synthesis of thymonucleic acid in normal and tumour cells by therapeutic doses of X-radiation, and the reality of indirect inhibiting effects on a second shielded tumour in the same animal. Another relevant application of P^{32} is the work of Brues, Tracy and Cohn (1944), who showed that, while the turnover of thymonucleic acid phosphorus in non-growing liver is extremely slow, rapid synthesis of thymonucleic acid occurs in regenerative and neoplastic

TABLE I.—*Some Stable and Radio-active Isotopes of Medical and Biological Interest.*

Element.	Isotope.	Stability or half-life.	Per cent abundance of stable isotope.	Type of radiation.	Energy of radiation in Mev.	
					β Particles.	γ Rays.
Hydrogen	H^1	Stable	99·98	..	..	..
	H^2	„	0·02	..	..	..
	H^3	31 ± 8 yr.	..	β−	0·015	..
Carbon	C^{11}	20·4 min.	..	β+	0·98	..
	C^{12}	Stable	98·9	..	..	..
	C^{13}	„	1·1	..	..	..
	C^{14}	~5000 yr.	..	β−	0·145	..
Nitrogen	N^{13}	9·93 min.	..	β+, γ	0·92, 1·20	0·28
	N^{14}	Stable	99·62	..	..	..
	N^{15}	„	0·38	..	..	..
	N^{16}	7·3 sec.	..	β−	10 ± 0·5	..
Oxygen	O^{15}	2·1 min.	..	β+	1·7	..
	O^{16}	Stable	99·76	..	..	..
	O^{17}	„	0·04	..	..	..
	O^{18}	„	0·20	..	..	..
	O^{19}	31 sec.	..	β−	~3·3	..
Fluorine	F^{18}	112 min.	..	β+	0·7	..
	F^{19}	Stable	100·0	..	..	..
Sodium	Na^{22}	3·0 yr.	..	β+, γ	0·575	1·30
	Na^{23}	Stable	100·0	..	..	..
	Na^{24}	14·8 hr.	..	β−, γ	1·39	1·38, 2·76
Magnesium	Mg^{24}	Stable	77·4	..	..	..
	Mg^{25}	„	11·5	..	..	..
	Mg^{26}	„	11·1	..	..	..
	Mg^{27}	10·2 min.	..	β−, γ	1·8	0·64, 0·84, 1·02
Phosphorus	P^{30}	2·55 min.	..	β+	3·5	..
	P^{31}	Stable	100·0	..	..	..
	P^{32}	14·3 days	..	β−	1·71	..
Sulphur	S^{32}	Stable	95·1	..	..	..
	S^{33}	„	0·74	..	..	..
	S^{34}	„	4·2	..	..	..
	S^{35}	87·1 days	..	β−	0·17	..
	S^{36}	Stable	0·016	..	..	..
Chlorine	Cl^{34}	33 min.	..	β+, γ	2·4, 5·1	~3·4
	Cl^{35}	Stable	75·4	..	..	..
	Cl^{36}	~10^6 yr.	..	β−	0·66	..
	Cl^{37}	Stable	24·6	..	..	..
	Cl^{38}	37 min.	..	β−, γ	1·1, 2·8, 5·0	1·65, 2·15
Potassium	K^{39}	Stable	93·38	..	..	..
	K^{40}	$1·4 \times 10^9$ yr.	0·012	β−, γ	1·35	~2
	K^{41}	Stable	6·61	..	..	..
	K^{42}	12·4 hr.	..	β−	3·5	(?)

TABLE I (continued).—*Some Stable and Radio-active Isotopes of Medical and Biological Interest.*

Element.	Isotope.	Stability or half-life.	Per cent abundance of stable isotope.	Type of radiation.	Energy of radiation in Mev.	
					β Particles.	γ Rays.
Calcium	Ca^{40}	Stable	96·96	..	..	..
	Ca^{41}	8·5 days	..	K, γ, e−	..	1·1
	Ca^{42}	Stable	0·64	..	..	..
	Ca^{43}	„	0·15	..	..	..
	Ca^{44}	„	2·06	..	..	..
	Ca^{45}	180 days	..	β−, γ	0·3	~0·7
	Ca^{46}	Stable	0·0033	..	..	..
	Ca^{48}	„	0·19	..	..	..
	Ca^{49}	2·5 hr.	..	β−, γ	2·3	0·8
Iron	Fe^{52}	8·9 min.	..	β+	..	..
	Fe^{54}	Stable	5·9	..	..	..
	Fe^{55}	~ 4 yr.	..	K, e−	..	(0·006)
	Fe^{56}	Stable	91·7	..	..	..
	Fe^{57}	„	2·1	..	..	..
	Fe^{58}	„	0·3	..	..	..
	Fe^{59}	44 days	..	β−, γ	0·26, 0·46	1·30, 1·10
Bromine	Br^{79}	Stable	50·6	..	..	..
	Br^{80}	4·4 hr.	..	I.T.e−, γ	..	0·037
	Br^{81}	Stable	49·4	..	..	..
	Br^{82}	34 hr.	..	β−, γ	0·465	0·547, 0·787, 1·35
	Br^{83}	2·4 hr.	..	β−	~1·0	..
	Br^{84}	33 min.	..	β−, γ	5·3	..
Iodine	I^{124}	4·0 days	..	β+	..	..
	I^{126}	13 days	..	β−, γ	~1·1	~0·5
	I^{127}	Stable	100	..	..	..
	I^{128}	25·0 min.	..	β−, γ	2·02 (93%) 1·59 (7%)	0·428
	I^{129}	Very long	..	β−	..	..
	I^{130}	12·6 hr.	..	β−, γ	0·61, 1·03	0·417, 0·537, 0·667, 0·744
	I^{131}	8·0 days	..	β−, γ, e−	0·595	0·367, 0·080
	I^{132}	22·0 hr.	..	β−, γ	~1·3	~0·55
	I^{135}	6·7 hr.	..	β−, γ	~1·35	~1·6

β− = negative beta particle emitted from nucleus.
β+ = positive beta particle (positron).
γ = gamma ray.
e− = internal conversion electron.
I.T. = isomeric transition.
K = K-electron capture.

growth. An interesting possible diagnostic application of measurements of P^{32} in breast tumours in situ has been reported by Low-Beer, Bell, McCorkle, Stone, Steinbach and Hill (1946).*

The application of P^{32} as a tracer in metabolic investigations exemplifies the problems raised by this method of investigation. In order to avoid short-term effects, it is essential to calculate the dose of radiation received by the tissues from the radio-active isotope. For example, with P^{32}, one microcurie per g.

* One line of metabolic tracer investigation insufficiently well known is the use of radio-active brominated compounds, e.g. cholesterol dibromide (Friedman, Solomon and Werthessen, 1939).

TABLE II.—*Isotopes for Use as Radio-active Tracers. Produced by Means of the Pile.*

Isotopes.	Reaction.	Type of radiation.	Half-life.	Energy of radiation in Mev.	
				β Particles.	γ Rays.
H^3	$H^2 + n$[1] / $Li^6 + n$	β −	31 ± 8 years	0·015	..
C^{14}	$N^{14} + n \rightarrow C^{14} + p$	β −	5300 (± 15%) years / 4700 (± 10%) „	0·145	..
Na^{24}	$Na^{23} + n$	β −, γ	14·8 hours	1·39	1·38, 2·76
P^{32}	$P^{31} + n$	β −	14·3 days	1·71[3]	..
S^{35}	$S^{34} + n$	β −	87·1 „	0·17	..
Cl^{36}	$Cl^{35} + n$	β −	~10^6 years[3]	0·66	..
Ca^{45}	$Ca^{44} + n$	β −, γ	180 days	0·3	~0·7
Sc^{46}	$Sc^{45} + n$	β −, γ, K	85 „	0·26 / 1·5	1·25
Cr^{51}	$Cr^{50} + n$	K, e−, γ	26·5 „	—	0·32
Ti^{51}	$Ti^{50} + n$	β −, γ	72 „	0·36	1·0′
Fe^{59}	$Fe^{58} + n$	β −, γ	44 „	0·26 / 0·46	1·30 / 1·10
Ni^{59}	$Ni^{58} + n$	β +	15 years	0·05	..
Co^{60}	$Co^{59} + n$	β −, γ	5·3 „	0·31	1·30 / 1·10
Cu^{64}	$Cu^{63} + n$	β −, β +, K	12·8 hours	β − 0·58 / β + 0·66	..
Zn^{65}	$Zn^{64} + n$	K, β +, γ, e−	250 days	β + 0·4	1·14
Zn^{69}	$Zn^{68} + n$	I.T. γ	13·8 hours	1·0	0·44
Se^{75}	$Se^{74} + n$	K, e−, γ	125 days	..	0·18, 0·35
As^{76}	$As^{75} + n$	β −, β +, K, γ	26·8 hours	1·1, 1·7, 2·7	0·57, 1·25
Br^{82}	$Br^{81} + n$ (F.P.)	β −, γ	34 „	0·465	0·547 / 0·787 / 1·35
Br^{83}	F.P.	β −	2·4 „	~1·0	..
Rb^{86}	$Rb^{85} + n$	β −	19·5 days	1·60	..
Sr^{89}	$Sr^{88} + n$ / $U^{235} + n$	β −	55 „	1·5	No γ
Sr^{90}	F.P.	β −	25 years	0·6	No γ
Zr^{95} / Cb^{*95} (4)	$U^{235} + n$ / $Zr^{95} \rightarrow Cb^{*95} + \beta -$	β −, γ	65 days	0·39 (98%) / 1·0 (2%)	0·73 / ? 0·92
Mo^{99}	$Mo^{98} + n$; (F.P.)	β −, γ	67 hours	1·4	0·24, 0·75
Ru^{103}	$Ru^{102} + n$; F.P.	β−, γ	42 days	0·2 (95%) / 0·8 (5%)	0·56

TABLE II (continued).—*Isotopes for Use as Radio-active Tracers.*

Produced by Means of the Pile.

Isotopes.	Reaction.	Type of radiation.	Half-life.	Energy of radiation in Mev.	
				β Particles.	γ Rays.
$Ag^{108, 110}$	$Ag^{107, 109} + n$	K, e−, γ	225 days	1·3	0·6 0·9
Sb^{124}	$Sb^{123} + n$	β −, γ	60 „	2·45 0·74	1·72
I^{131}	$U^{235} + n$	β −, γ	8·0 days	0·595	0·367 0·080
Cs^{134}	$Cs^{133} + n$	β −, γ	1·7 years	0·75	0·8
Cs^{137}	$U^{235} + n$	β −, γ	33 „	0·5 (50%) 0·8 (80%)	0·75
Ba^{140}	$U^{235} + n$	β −, γ, e−	12·8 days	1·05	0·542
La^{140}	$Ba^{140} \rightarrow La^{140} + \beta -$	β −, γ	40·2 hours	0·90 (20%) 1·40 (70%) 2·12 (10%)	0·335 (1%) 0·49 (7%) 0·83 (14%) 1·63 (74%) 2·3 (4%)
Ta^{182}	$Ta^{181} + n$	β −, γ	97 days	0·53	1·22, 1·13[5]
W^{185}	$W^{184} + n$	β −, γ	77 „	0·6	?
Au^{198}	$Au^{197} + n$	β −, γ	2·7 days	0·8	2·5 0·44 0·28
$Hg^{203, 205}$	$Hg^{202, 204} + n$	β −, γ	51·5 „	0·3	0·28
Tl^{206}	$Tl^{205} + n$	β −	3·5 years	0·87	..
Bi^{210} (RaE)	$Bi^{209} + n$	β −	5·0 days	1·17	..

(1) The growth of H^3 in a heavy water pile is a natural by-product.

(2) Mean energy of β particles approximately 0·70 Mev.

(3) The data for Cl^{36} are rather uncertain.

(4) Cb^{*95} is a metastable state of half-life 35 days of the stable isotope Cb^{95}. It comes into transient equilibrium with Zr^{95}.

(5) Complex.

NOTE.—*Fast Neutron Irradiation within Reacting Core of Pile*:—The following isotopes are amongst those that can be produced in all probability in small amounts: C^{11}, Fe^{55}, Fe^{59}, As^{74}, Y^{88}.

β− = negative beta-particle. β+ = positive beta-particle (positron). γ = gamma ray. α = alpha-particle. e− = internal conversion electron. K = K-electron capture. I.T. = isomeric transition. F.P. = fission product.

of tissue delivers 43 r. per day, so that for many experiments one must not exceed tracer doses corresponding to 1/20 microcurie per g. of animal with times of observation of the order of 6 hours.

Of especial interest is the work of Abels, Kenney, Craver, Marinelli and Rhoads (1941), who accidentally found significant metabolic changes in the leucocytes in chronic leukaemias after total body X-radiation with doses as small as 3 r., as a result of the radiation given by sub-therapeutic doses of P^{32}.

Of much greater importance in the clinical applications of isotopic tracer research is the avoidance of all possible risk of carcinogenesis as a long-term result of the radio-active material introduced. In a recent report on the "Health Protection Activities of the Plutonium Project," Dr. Robert S. Stone (1946) states that "it is now well established that (radio)strontium given to mice and rats in amounts insufficient to kill them for many months can cause bone and lymphatic tissue tumours to develop in significant numbers of animals. . . . Over long periods of time, plutonium has been shown to cause atrophy of the bone and bone sarcomas." It is evident that great caution is necessary before introducing any radio-active materials into human subjects. However, it is likely that for many isotopes safe conditions for tracer work will be established after dosimetric studies and prolonged animal experiments. One factor of importance is the usually long latent period of carcinogenesis by radiations and radio-active substances.

Radio-active sources in radiotherapy.

The possibility of practical artificial radio-active sources for use in radiotherapy has been discussed in the two previous papers (Mitchell, 1946 *a* and *b*). A revised list of isotopes made in the pile and suitable for use as gamma ray sources in radiotherapy is given in Table III.

The most promising substitute for radium is considered to be radio-cobalt, Co^{60}, of half-life 5·3 years. The second choice is probably Ta^{182}, of half-life 97 days.

Unseparated fission products contained in uranium metal irradiated in the pile should be investigated as a possible gamma-ray source. Large amounts of separated fission products are likely to be available, and those listed in Table III appear to be perhaps the most suitable.

The gamma radiation from radio-cobalt, Co^{60}, appears to be eminently suitable for radio-therapeutic application, being approximately monochromatic and of slightly higher mean energy (1·2 Mev.) than the mean energy of the usual filtered gamma radiation from radium (approximately 0·8 Mev.). The accompanying beta radiation is relatively soft and easily removed by filtration. It has been calculated by Professor W. V. Mayneord (March 1, 1946—unpublished) that the dose rate at 1 cm. from a point source of 1 millicurie of Co^{60} enclosed in a platinum envelope of thickness 0·5 mm. is 11·1 r. per hour. However, it may prove more convenient to measure Co^{60} sources in terms of equivalent radium.

The production of large amounts of these isotopes by means of the pile raises the practical possibility of the therapeutic application of large gamma ray sources—"mass radiation units" equivalent to 50–100 g. of radium.

Of limited therapeutic interest is the possibility of development of beta ray applicators, using P^{32} or Sr^{89}. Low-Beer (1946) has published a report on human skin reactions produced by the external use of radio-phosphorus, applied

TABLE III.—*Radio-active Isotopes for Use in Radiotherapy as Gamma-ray Sources Produced by Means of the Pile.*

(The isotopes are listed in descending order of probable suitability as substitutes for radium. Unseparated fission products must be investigated as an alternative.)

	Isotopes.	Reaction.	Type of radiation.	Half-life.	Energy of radiation in Mev.		Number of γ rays per disintegration.
					β Particles.	γ Rays.	
1.	Co^{60}	$Co^{59} + n$	$\beta-, \gamma$	5·3 years	0·31	1·30 1·10	2
2. (*a*)	Ta^{182}	$Ta^{181} + n$	$\beta-, \gamma$	97 days	0·53	1·22 1·13	<1
(*b*)	Sc^{46} [1]	$Sc^{45} + n$	$\beta-, \gamma$, K	85 „	0·26 1·5	1·25	<1
(*c*)	Sb^{124}	$Sb^{123} + n$	$\beta-, \gamma$	60 „	2·45 0·74	1·72	<1
3.	Fission Products.						
(*a*)	Zr^{95} Cb^{*95} [2]	$U^{235} + n \rightarrow$ $Zr^{95} \rightarrow Cb^{*95} + \beta-$	$\beta-, \gamma$	65 days	0·39 (98%) 1·0 (2%)	0·73 ? 0·92	~1
(*b*)	Ba^{140}	$U^{235} + n \rightarrow$	$\beta-, \gamma, e-$	12·8 days	1·05	0·542	—
	La^{140}	$Ba^{140} \rightarrow La^{140} + \beta-$	$\beta-, \gamma$	40·2 hours	0·90 (20%) 1·40 (70%) 2·12 (10%)	0·335 (1%) 0·49 (7%) 0·83 (14%) 1·63 (74%) 2·3 (4%)	~1

(1) It is doubtful whether adequate supplies of Scandium could be obtained.

(2) Cb^{*95} is a metastable state of half-life 35 days of the stable isotope Cb^{95}. It comes into transient equilibrium with Zr^{95}.

in the form of Na_2HPO_4 solution soaked in blotting-paper and dried. In addition to therapeutic applications, these beta-ray sources may be useful in experimental cancer research, e.g. Stone (1946) states that "it has been established that single large doses of beta rays and multiple small doses can cause cancers in the skin without the animal being killed by the effect of the beta rays."

Therapeutic possibilities of selectively concentrated radio-active agents.

Radio-active isotope therapy has as yet been limited to inorganic compounds. Radio-phosphorus (P^{32}), and to a much smaller extent the radio-iodines (I^{130} and I^{131}), are the only isotopes with which limited clinical trials appear justified without further preliminary animal experiments. In general it seems unlikely that inorganic radio-active isotope therapy will stand the test of time. It seems much more hopeful to study the selective concentration by malignant cells of suitable organic compounds carrying radio-active isotopes of many elements (Rhoads, 1946).

The usual therapeutic applications of P^{32} in the form of Na_2HPO_4 appear to depend upon its synthesis into nucleic acids by the multiplying cells. One other method of therapeutic application of P^{32} reported (Jones, Wrobel and Lyons, see Low-Beer, Lawrence and Stone, 1942) depends on the selective concentration of colloidal anhydrous chromic phosphate by the cells of the reticulo-endothelial system. It is easy to understand the concentration of the radio-iodines by the thyroid in hyperthyroidism and in the rare cases of carcinoma of the thyroid, where the primary and sometimes also the metastases retain the function of secretion. One must be sceptical of the therapeutic possibilities in bone sarcoma of Sr^{89} or Ca^{45}; it is interesting to note that Stone (1946) reports that radio-"strontium, barium, zirconium, yttrium and others locate quite selectively in the bones, and many of them stay for long periods of time."

Radio-phosphorus, P^{32}, has been used since 1936 mainly in U.S.A., in the treatment of patients with chronic myeloid and lymphatic leukaemia, polycythemia vera, lymphosarcoma and various related diseases (Low-Beer, Lawrence and Stone, 1942; Kenney, 1942; Reinhard, Moore, Bierbaum, Moore and Kamen, 1946). Lindgren (1944) showed that P^{32}, which is selectively concentrated, gave better results than Na^{24}, which is not selectively concentrated. The therapeutic possibilities and dosage of radio-phosphorus has been reviewed recently (Mitchell, 1947*b*). It is considered that the present position with regard to its therapeutic applications may be summarized as follows:

1. In the treatment of chronic myeloid and chronic lymphatic leukaemia, P^{32} is probably as satisfactory as, but no better than X-radiation. It is important to investigate the possibility of development of methods of routine treatment of these diseases by means of P^{32}. On the average, each case is likely to require 10 to 15 millicuries of P^{32} given, preferably intravenously, in the form of a solution of Na_2HPO_4 in fractions in an over-all time of 10–12 weeks. It is desirable to correlate the dose with roentgen units (Marinelli, 1942), and to measure the differential absorption ratio in different tissues (Kenney, Marinelli and Woodard, 1941). The greatest risk to be avoided is bone marrow damage.

2. Radio-phosphorus is stated to be the treatment of choice in polycythaemia vera, but even here its action may be too slow, and ancillary venesection frequently required.

TABLE IV.—*Production of Selected Isotopes by Means of the Cyclotron.*

Radio-element.	Type of radiation.	Half-life.	Energy of radiation in Mev.		Approximate yield[1] in microcuries per micro-ampere hour.	Produced by[2]
			β Particles.	γ Rays.		
H^3	β−	31 ± 8 years	0·015	..	0·1	Be–d–H^3
C^{11}([6])	β+	20·4 min.	0·98	..	500	B–d–n
C^{14}	β−	5300 (± 15%) years 4700 (± 10%) „	0·145	..	0·00005	N–n–p
N^{13}([3])	β+, γ	9·93 min.	1·20, 0·92	0·28	1000	C–d–n
Na^{22}	β+, γ	3·0 years	0·575	1·30	1	Mg–d–α
Na^{24}	β−, γ	14·8 hours	1·39	1·38, 2·76 ([4])	10	Na–d–p
P^{32}	β−	14·3 days	1·71	No γ	200	P–d–p
S^{35}	β−	87·1 „	0·17	..	0·1	S–d–p
Ca^{45}	β−, γ	180 „	0·3	~0·7	0·01	Ca–d–p
Mn^{54}	K, γ	310 „	..	0·85	1	Fe–d–α
Fe^{55}	K, e−	~4 years	..	(0·006)	..	Fe–d–p
Fe^{59}	β−, γ	44 days	0·26; 0·46	1·30; 1·10	0·03	Fe–d–p
Cu^{64}	β−, β+, K	12·8 hours	β− 0·58 β+ 0·66	No γ	3000	Cu–d–p
Zn^{65}	β+, K, γ, e−	250 days	β+ 0·4	1·14	0·5	Cu–d–2n
As^{74}	β−, β+, γ	16 „	β− 1·3 β+ 0·9	0·582	2	Ge–d–n
As^{76}	β−, β+, K, γ	26·8 hours	1·1, 1·7, 2·7	0·57, 1·25	10	As–n–γ (Be–d–n)
Br^{82}	β−, γ	34 „	0·465	0·547 0·787 1·35	10	Br–n–γ (Be–d–n)
Rb^{86}	β−, γ	19·5 days	1·60	..	10	Rb–d–p
Sr^{89}	β−	55 „	1·5	No γ	7	Sr–d–p
I^{120}	β−, γ	12·6 hours	0·61, 1·03	0·417, 0·537, 0·667, 0·744	~200 ([5])	Te–d–2n
I^{121}	β−, γ	8·0 days	0·60	0·367 0·080	20	Te–d–n
85^{211}	K, α	7·5 hours	5·94 (α)	..	1	Bi–α–2n (32 Mev. α's)

([1]) The yields with the cyclotron are taken from the table given by J. G. Hamilton (*Radiology*, 1942, **39**, 570), and are included to enable comparison with production by means of the pile.

([2]) This column specifies in order the nucleus bombarded, the particle used for bombardment and the particle emitted.

([3]) Data for production in pile are not available.

([4]) See Table II.

([5]) Immediately after a short bombardment of Te with 14 Mev. deuterons (Chapman and Evans, 1946).

3. It seems likely that the only other disease, or group of diseases, in which the therapeutic possibilities of P^{32} administered internally should be investigated, is lymphosarcoma.

One interesting aspect of the present position is that, while cases of Hodgkin's disease do not respond as favourably to P^{32} as to X-radiation, promising results are being obtained in this condition, perhaps especially in the radio-resistant stages with the "nitrogen mustards." (References—General.)

The therapeutic possibilities of the radio-iodines must be considered with great caution. A considerable number of clinical trials have been carried out in the U.S.A. This work has been reviewed by Leucutia (1946). It is evident from the recent papers of Hertz and Roberts (1946), and Chapman and Evans (1946), that selected cases of hyperthyroidism may respond satisfactorily to radio-iodine therapy. However, it seems that as yet there is insufficient evidence as to dosage and long-term effects of the radio-iodines to exclude with certainty the possibility under some conditions of late carcinogenesis. There is also the further question of possible renal damage by the excreted radio-iodine. Until further information on these subjects is available, it is probably wise to restrict clinical therapeutic trials of the radio-iodines to the much more difficult problem of carcinoma of the thyroid.

Only a very small proportion of all cancers of the thyroid retain the function of iodine concentration; of these only a few concentrate radio-iodines sufficiently to deliver therapeutically effective doses. However, there are the extremely rare cases of carcinoma of the thyroid, where functional secretion is a striking feature of both the primary and metastases, and where dramatic results can be obtained by radio-iodine therapy (Seidlin, Marinelli and Oshry, 1946). Accordingly the present position appears to be that there is only a very limited field for clinical trials of the therapeutic possibilities of the radio-iodines.

Therapeutic applications of high energy beta and gamma radiations.

Considerable progress in the development of high energy generators has taken place recently since the previous short review (Mitchell, 1946*a*), which this paragraph supplements.

The synchrotron has been developed as a practical instrument (Goward and Barnes, 1946), and should become available in this country as a source of gamma radiation of energy up to 30 Mev. for dosimetric and biological investigations, and subsequent clinical trials in the treatment of deeply situated malignant tumours. A large number of papers have recently appeared on the theory of the synchrotron, e.g. Bohm and Foldy, 1946. The synchrotron can be operated initially as a betatron until the electrons have reached an energy of perhaps 1·5 Mev., when the dee voltage is turned on and the machine works as a synchrotron for the rest of the acceleration.

Kerst and his collaborators (Skaggs, Almy, Kerst and Lanzl, 1946) have succeeded in extracting the electron beam from the betatron, but the therapeutic possibilities of these high energy beta particles are rather uncertain.

It now appears likely that there will be very little therapeutic superiority of the high energy gamma radiation from a betatron or synchrotron operated at 50 Mev. over that for 20 Mev. Probably the highest energy required for clinical therapeutic trials will be provided by an instrument working up to 30 Mev.

SUMMARY.

This review summarizes recent information on the following subjects :

1. Properties and production of isotopes of interest, mainly radio-active, with Tables.

2. Isotopic tracer research, with precautions necessary, including avoidance of risk of carcinogenesis in possible clinical applications.

3. Artificial radio-active sources for use in radiotherapy made by means of the pile (see Table III). The most promising substitute for radium appears to be radio-cobalt, Co^{60}, of half-life 5·3 years. Unseparated fission products should be investigated as an alternative.

4. Therapeutic possibilities of selectively concentrated radio-active agents, including the present position with regard to therapeutic applications of radio-phosphorus and the radio-iodines.

5. Therapeutic application of high energy (30 Mev.) gamma radiation and the development of the synchrotron.

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INVASION OF THE INTERNAL MAMMARY LYMPH GLANDS IN CARCINOMA OF THE BREAST.

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ATTEMPTS to find some reliable basis for prognosis in carcinoma of the breast have proved disappointing. Several factors have been thought valuable in formulating a prognosis, chief among which have been the presence or absence of secondary deposits in the axillary lymph nodes and the histological character of the growth. But it has been found that, even in cases with no axillary involvement and carcinomata of low histological malignancy, there has been a mortality from recurrence of about 25 per cent in the first five years after operation. If the axillary lymphatics were the only path by which carcinoma cells could escape from the breast, this mortality would be inexplicable. The axilla is not, however, the only path, and we set out below histological proof that the internal mammary glands may often be invaded at the same time as, and sometimes before, carcinoma has reached the axilla.

MATERIAL AND METHODS.

The operative specimens from five radical mastectomies and the corresponding five internal mammary glands, removed from the second intercostal space of the affected side, were studied microscopically. A biopsy specimen from one case was also used. In the first case the internal mammary gland was removed post-mortem in 1938 from a patient who died eleven days after a radical mastectomy. The last four cases were a recent consecutive series, operated on by one of us (R.S.H.), in which the internal mammary gland was excised at the same time as radical mastectomy was performed.

Case histories.

Case 1.—Msx. P.M. 221/38. Aged 64. She had noted a lump in her left breast for 14 weeks, and was admitted to the Middlesex Hospital under the care of the late Mr. E. Pearce-Gould. Left radical mastectomy was performed. A known hypertensive, she had a stormy post-operative course, with early collapse and subsequent anuria, and she died eleven days after the operation. The operative specimen showed a small polygonal cell carcinoma with deposits in the axillary glands. At post-mortem a small deposit was found in the right lung, and the left second intercostal space contained a gland invaded by carcinoma histologically similar to the primary growth.

Case 2.—Msx. Reg. No. B32553. Aged 76. A left radical mastectomy, presumably for carcinoma, had been performed elsewhere 18 years previously. The patient had noted a lump in the right breast for six months. A right radical mastectomy was performed for an obvious clinical carcinoma, and the second space internal mammary gland on the right side was also removed. The primary

growth and the internal mammary gland both showed polygonal cell carcinoma, but the axillary glands were free from growth.

Case 3.—Msx. Reg. No. 23806. Aged 56. A lump had been noticed in the right breast for three months. A right radical mastectomy was performed, the right second space internal mammary gland also being removed. The primary tumour was proved on section to be a carcinoma, but both the axillary and internal mammary glands were free from growth.

Case 4.—Msx. Reg. No. B34643. Aged 62. A lump had been present in the left breast for two years. A left radical mastectomy was performed and the left second space internal mammary gland was also removed. The primary tumour was proved on section to be a carcinoma, and both axillary and internal mammary glands were invaded by similar growth.

Case 5.—A private patient, aged 48, had known that a small lump had been present in her left breast for 18 months. It was very mobile, and was excised in the belief that it was a fibrous nodule. Section, however, showed a carcinoma. Fourteen days later a left radical mastectomy was performed and the left second space internal mammary gland was also removed. Neither the breast nor the axillary glands showed any evidence of growth, but the internal mammary gland contained a small deposit of carcinoma (see Fig. 1 and 2).

The last four cases made uninterrupted recovery from operation.

Anatomy of the internal mammary lymphatic chain.

The best description of the internal mammary lymphatic chain is that of Stibbe (1918). One or two glands are found fairly constantly in each of the three upper intercostal spaces, embedded in the loose fat which lies deep to the internal intercostal muscle. The second space gland is the most constant, and Stibbe found it in 96 per cent of the 60 subjects he examined. The internal mammary vessels also lie in this fat in a plane posterior to the glands and usually about half an inch lateral to the edge of the sternum. The glands may be either medial or lateral to the vessels. A thin but strong sheet of fascia, the costo-sternal fascia, intervenes between the glands and vessels and the pleura in the upper two spaces, and the transversus thoracis muscle in the lower spaces. The lymph glands show a tendency to atrophy and fatty replacement as age advances. Fig. 3 and 4 summarize the salient anatomical points.

Operative removal of the second space lymph gland.

The external intercostal membrane and the internal intercostal muscle are reflected as a somewhat ragged flap, either from lateral to medial and hinged on the inner end of the intercostal space : or from medial to lateral and hinged an inch lateral to the edge of the sternum. We prefer the former method, since bleeding from the perforating branch of the internal mammary artery seems easier to control. The obliquely running fibres of the intercostal musculature are so loosely bound together that this flap cannot be neatly sutured back into position at the end of the operation. Once the intercostal flap has been reflected, dissection should proceed with non-toothed dissecting forceps only. The internal mammary vessels are usually well seen and act as a guide. The lymph glands may be difficult to find, especially if they are not invaded by growth, but any tissue removed should be sectioned. In Case 5 a tiny piece of somewhat transparent tissue was excised and thought to be fat, but the microscope revealed an

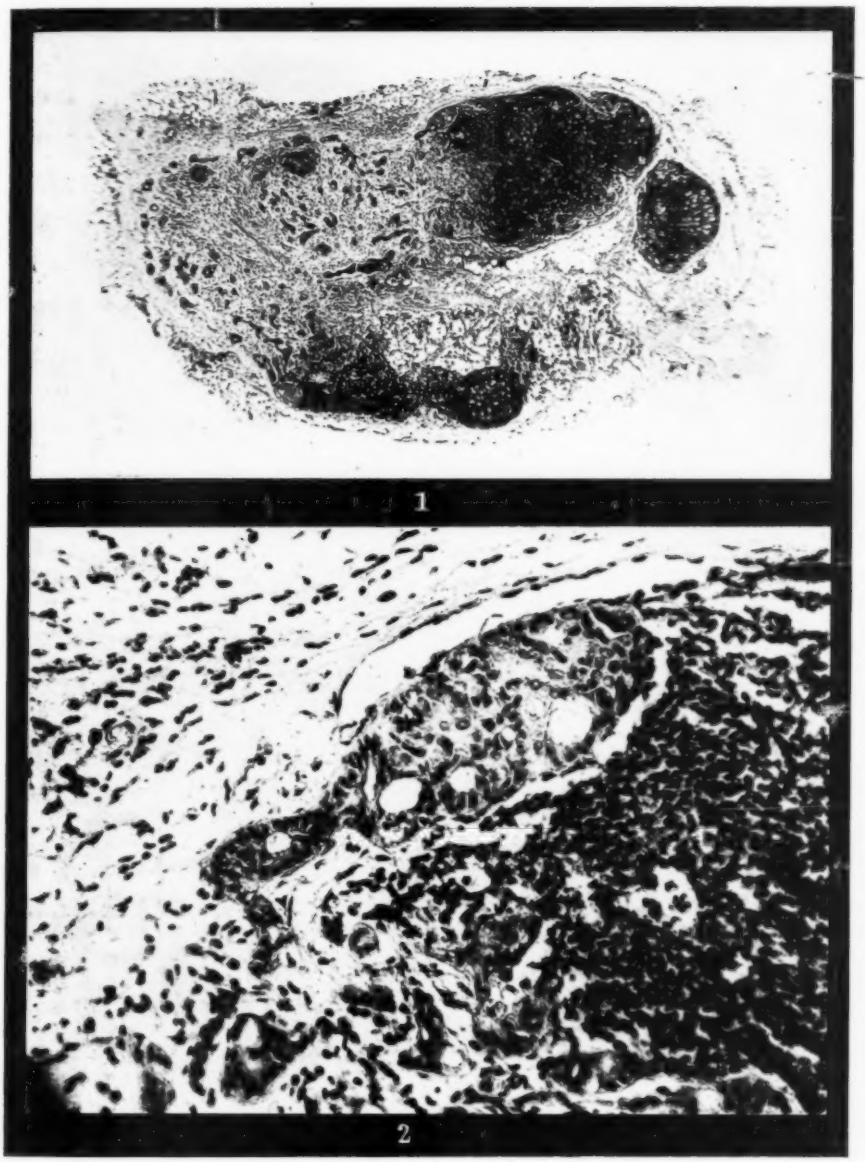

FIG. 1.—Section of the internal mammary gland from Case 5. (× 40.)
FIG. 2.—Section of the internal mammary gland from Case 5. (× 400.)

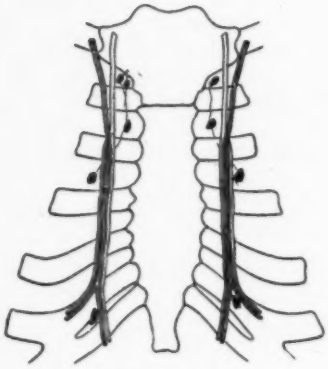

FIG. 3.

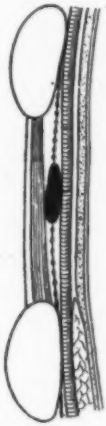

FIG. 4.

FIG. 3.—Diagram to illustrate the anatomy of the internal mammary glands. Seen from behind (after E. P. Stibbe).

FIG. 4.—Diagram to illustrate the anatomy of the internal mammary glands. Sagittal section through the antero-medial end of the second intercostal space (after E. P. Stibbe).

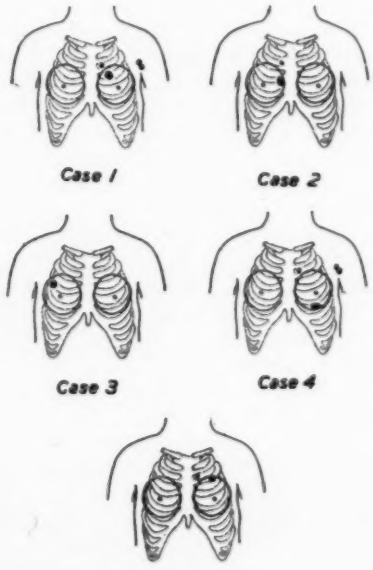

FIG. 5.—Diagram to show the sites of the primary tumours and the secondary deposits in the five cases described. The primary tumour, in all cases, is inside the circle of the breast, the secondary deposits outside the circle of the breast.

invaded lymph gland (Fig. 1 and 2). So far, the pleura has not been injured, but the anaesthetist should be warned that this accident is possible, so that he may have positive pressure immediately available. The internal mammary vessels have not yet been wounded. They might give rise to troublesome haemorrhage, but this should easily be controllable by packing while the main trunk was being ligated in the spaces above and below. Closure of the intercostal space is done as neatly as possible but is seldom complete. On one occasion a flap of pectoralis major was turned into the space, but it does not seem necessary to make a complete closure of the intercostal space if the suture line of the skin flap does not cross the deficiency.

RESULTS.

The primary breast tumour in all five cases was a polygonal cell carcinoma. In two patients both the axillary and internal mammary glands were invaded by growth. In two patients the internal mammary gland was invaded, but the axillary nodes were free. In one patient neither the axillary nor the internal mammary glands were invaded. Fig. 5 is a diagrammatic representation of the sites of the primary growth and of the glandular deposits.

DISCUSSION.

Although anatomists have known and taught for very many years that the internal mammary glands receive lymph from the breast, little attention has been paid to this knowledge. Proper investigation of the frequency with which these glands are invaded in carcinoma of the breast appears never to have been attempted, though their removal is technically quite easy. Halsted in 1898 wrote: "Dr. H. W. Cushing, my house-surgeon, has in three instances cleared out the anterior mediastinum on one side for recurrent cancer. It is very likely, I think, that we shall, in the near future, remove the mediastinal contents at some of our primary operations." This idea does not seem to have been followed up. Sampson Handley (1922) explored the anterior mediastinum in six cases of carcinoma of the breast, finding glands in two, both of which were the seat of metastasis. He thought at this time that the anterior mediastinal glands were not infected so early or so constantly as the axillary glands. But by 1927 he had accumulated further evidence of the frequency with which carcinomata recurred at the antero-medial ends of the intercostal spaces after radical operations. He described in detail a case in which recurrences appeared in the inner ends of the intercostal spaces, one after another from above downwards, in a patient who finally succumbed to the disease twelve years after a radical mastectomy; and he stated his belief that, by the time the axillary glands were enlarged, the internal mammary glands had frequently, and perhaps usually, been invaded. Scarff and Handley (1938) investigated the ten-year results of radical mastectomy in 172 cases of carcinoma of the breast. They found that patients without evidence of axillary deposits (the so-called Stage 1 cases) had a mortality of 25 per cent from recurrence in the first five years after operation, and that this mortality continued at about the same rate between the 5th and 10th years. They suggested that the principal point used to judge the clinical stage of a growth, namely the involvement or otherwise of the axillary nodes, was too arbitrary, and that the internal mammary glands were an important but unknown factor about which

no microscopic evidence was available. There do not appear to be any further specific case-records or evidence in the literature bearing on the subject of invasion of the internal mammary glands in carcinoma of the breast, though the vast mass of publications on the subject of mammary cancer makes a complete search wellnigh impossible.

The present series of cases, five in number, though it is too small to justify the drawing of broad conclusions, indicates that a very important point in the formulation of a prognosis, namely the state of the internal mammary glands, has received practically no attention whatever. It may prove to be the factor which explains the mortality in those patients whose axillae were clear at operation, and who for this reason had hitherto been expected to justify the word " cure." The addition of a second intercostal space biopsy adds little to the time or the shock of a radical mastectomy, and we suggest that it might well become part of the technique of the standard operation. The knowledge gained by the microscopic examination of the second space lymph gland in all cases would not only advance the science of pathology, but would give a wider basis, in the individual case, on which prognosis could intelligently be formulated and treatment by radiotherapy accurately directed.

The practical application of our findings to treatment requires further observation and thought. It is clearly not feasible surgically to excise the internal mammary chain *in toto*. Even if the intercostal spaces were attacked seriatim, invaded lymphatic vessels might be left posterior to the costal cartilages. The treatment of these glands must be by some form of radiotherapy. There seems much to commend Sampson Handley's method of burying radium tubes in the intercostal spaces at the time of operation, thus securing a localized but intense irradiation of those points where intensity is most needed.

It remains to discover whether, in a large series of cases, invasion of the internal mammary glands is as frequent as it would appear to be, what influence the site of a carcinoma in the breast has on internal mammary deposits, and what is the best method of treatment. The small number of cases does not lend itself to statistical analysis, and the collection of further data is now proceeding.

SUMMARY.

(1) In five unselected cases of carcinoma of the breast, a lymph gland of the internal mammary chain was removed through the second intercostal space at, or shortly after, radical mastectomy.

(2) In four cases the internal mammary gland was histologically proved to contain carcinoma. In only two cases were there axillary deposits. The case in which the internal mammary gland was free from growth also had no deposit in the axilla.

(3) The surgical anatomy of the internal mammary lymphatic chain and the operative removal of one of its glands is described.

(4) It is suggested that the technique of radical mastectomy might be modified to include removal of the second space internal mammary lymph gland. Microscopic examination of this gland might greatly increase the accuracy of prognosis, and prove of considerable assistance in post-operative treatment by irradiation.

Our thanks are due to the Director, The Bland Sutton Institute, The Middlesex Hospital, for permission to use the pathological reports; to Professor R. W.

Scarff for much helpful criticism; and to the Editor, *The Journal of Anatomy*, for permission to reproduce, with modifications, Fig. 2 and 3 from E. P. Stibbe's paper.

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SQUAMOUS CELL CARCINOMA OF THE BUCCO-PHARYNGEAL REGION.

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IN recent years many attempts have been made to correlate the histological appearances of new growths with their clinical behaviour and radio-sensitivity. These studies have disclosed the existence of a group of ill-differentiated highly malignant but equally highly radiosensitive buccopharyngeal carcinomata of uncertain histogenesis. While much attention has been paid to their morphology, the literature yields but scant information of value regarding the relative malignancy of these tumours. Varying nomenclature, inadequate follow-up, and an insufficiency of cases, render many of the views expressed little more than conjectures. The purpose of this paper is to elucidate further the natural history of this group. This we have attempted through an analysis of 408 cases of cancer in the neighbourhood of the mouth. The frankly keratinizing members of our series we have taken as a standard with which to compare the behaviour of our remaining histological types.

The morphological features of the more differentiated epitheliomata have been so completely evaluated that any further discussion would be superfluous. Duval and Lacassagne (1922) have probably provided the most complete classification of these growths, but made no attempt to correlate structure with potential malignancy. The most widely known study with this end in view is that of Broders (1920), who grouped the squamous cancers into four grades on the basis of cell differentiation. The anaplastic growths of Grade IV merit a brief description. They are characterized by their large, polygonal, clearly demarcated cells, which are distributed in groups of two or three, in sheets, columns, or as a diffuse infiltration. The cytoplasm is eosinophil in reaction, while the sharply cut, round or oval nucleus houses a coarse chromatin network with a prominent nucleolus. Such growths belong to that poorly differentiated group which has aroused such interest during the past two decades, a group which has become a

veritable pathologists' Babel. It is a heterogeneous assembly, including transitional cell carcinoma, lympho-epithelioma, and the non-keratinizing squamous cancers which we have just described.

"Lympho-epithelioma" was the term employed by both Régaud (in a paper by Reverchon and Coutard) and Schmincke in 1921 to describe what they regarded as being a specific group of highly radiosensitive tumours arising from the so-called pharyngeal lympho-epithelium. It was Ver Eecke in 1899 who first employed the latter term ("tissu lymphothélial") in a paper concerning the histogenesis of the frog's thymus. Jolly (1915) (working particularly with the bursa Fabricii of birds) and Mollier (1913) elaborated the concept in a series of papers, extending it to all tissues characterized by a covering epithelium infiltrated by underlying lymphoid deposits. So intimate and fundamental was this relationship, they believed, that such tissues should be regarded as a class apart and designated as such. Lympho-epithelium so defined is found in the faucial tonsils, the nasopharynx, laryngopharynx, base of tongue, thymus, and ileum. Since their first description in 1921 lympho-epitheliomata have been reported as arising from all these organs with the exception of the small intestine. Tonsillar growths earlier described as alveolar sarcomata and encephaloid epitheliomata were probably of this nature (Dietrich, 1926). In 1926 Jovin published a series of cases, drawing attention to the salient clinical features of the tumour and elaborating Regaud's morphological study. Three years later a paper by Ewing further established the neoplasm as a specific entity.

The tumours occur at all ages, but particularly in middle and late adult life, males appearing to be affected more often than females. The primary lesion grows slowly and tends to be clinically latent for a considerable period of time, especially if it is situated in a region such as the nasopharynx. It appears as a smooth or finely granular, firm, globular tumour, at first a mere nodule, but later attaining considerable dimensions. Infiltrative spread and superficial ulceration occur fairly late in the evolution of the growth. Cervical lymph glandular metastases appear at an early date, while the final phase is frequently ushered in by distant visceral extension, osseous deposits being rather characteristic (Kienbock and Selka, 1935; Ch'in and Szutu, 1940). The histological picture may be quite typical. Syncytia of large, palely staining cells appear as sheets, irregular clumps, or columns, in a lymphocytic stroma of varying density. The cell outlines are indistinct or quite indiscernible. The nuclei are large, round or oval, vesicular, and with prominent nucleoli. The appearance described by Schmincke is somewhat different. The epithelial cells are more discrete, and in many areas lie scattered independently, while there is a heavy lymphocytic infiltration throughout. Ewing was doubtful of the specificity of Schmincke's description, and found its distinction from other types of undifferentiated pharyngeal growth difficult or impossible.

The term "Transitional Cell Carcinoma" was first employed by Quick and Cutler (1927) to describe a group of highly radiosensitive buccopharyngeal tumours of uncertain histogenesis which lacked the classical features of epithelioma, but presented transitional epithelial characters. Ewing's writings (1929, 1940) have done much to clarify the morphological features of this growth. The most frequently encountered sites are the tonsil, base of tongue, nasopharynx, laryngopharynx, nasal cavity, and accessory nasal sinuses. Quick and Cutler described the primary lesion as possessing "a finely granular, velvety surface,

which looks like an erosion of the mucous membrane rather than a frank ulceration. The lesion . . . gives the impression of having originated in the deeper structures and adhered to and eroded the mucous membrane from beneath." This description is, however, far from being specific. The clinical course resembles that of lympho-epithelioma—a small and often occult primary focus, and early cervical lymph glandular metastasis. Histologically, transitional cell cancer is typified by the presence of broad, clearly demarcated, epithelial columns and alveolar formations. The cells are cylindrical, polygonal or spindle-shaped, with a rather hazy cytoplasmic membrane. The oval or fusiform nucleus is more chromatic than that of lympho-epithelioma, while its nucleolus is less distinct. Frequently the marginal cells appear regimented as though forming a basal layer. Degeneration and necrosis of the central zones of some of the epithelial formations may be observed. Lymphocytic infiltration of the stroma may be present, but is never marked.

There is considerable divergence of opinion regarding the justification of the classification of lympho-epithelioma and transitional cell carcinoma as specific entities. Ewing (1940) accepts the distinction, but admits that when atypical they may be quite indistinguishable from each other and from other ill-differentiated epithelial and mesoblastic growths. The majority of the French authors employ the term "lympho-epithelioma" to embrace both tumours (Jovin, 1939). Harvey, Dawson and Innes (1937) believe that the descriptions cover two types of growth—squamous cell carcinoma and reticulum-cell or lympho-sarcoma. Cappell (1938) could not make an absolute distinction between lympho-epithelioma and transitional cell cancer.

MATERIAL.

The material at our disposal consists of 408 cases of carcinoma of the buccal cavity, pharynx, larynx, and accessory nasal sinuses treated at the Middlesex Hospital between the years 1930 and 1939. Each has been traced to death or for a minimum period of five years following the first examination. For purposes of classification we have created the following groups according to the site of the primary lesion:

1. Upper and lower lips and commissures (purely cutaneous growths have been excluded), 2. Larynx proper. 3. Tongue (excluding base). 4. Floor of mouth. 5. Upper and lower alveoli, cheek, palate, and anterior pillars of fauces. 6. Faucial tonsil, and base of tongue. 7. Laryngopharynx, nasopharynx, and posterior oropharyngeal wall. 8. Nasal cavity and accessory nasal sinuses. 9. Cases in which cervical metastases were present in the absence of a clinically demonstrable primary lesion. We have distinguished six histological groups: I, Frankly keratinizing squamous cell carcinoma (Fig. 2 and 3); II, early keratinizing squamous cell carcinoma (Fig. 4 and 5); III, anaplastic squamous cell carcinoma (Fig. 6 and 7); IV, transitional cell carcinoma (Fig. 8 and 9); V, lympho-epithelioma (Fig. 10 and 11); VI, undifferentiated carcinoma (Fig. 12 and 13). Group I corresponds approximately to Broders' (1926) Grades I and II, Group II to his Grade III and part of IV, and Group III to the least differentiated residue of Grade IV. We have followed Ewing (1929) in including in Group IV the transitional-like growths arising from the pseudo-stratified columnar epithelium of the nose and accessory nasal cavities (the so-called Schneiderian

carcinoma, and the solid cylindrical cell carcinoma of Ringertz (1938)). We have restricted the group, lympho-epithelioma, to tumours of the Régaud type. Our undifferentiated group corresponds to that of Ringertz. The cells are distributed in solid masses or diffuse infiltrations. The cell body is spheroidal or polygonal in form with palely staining cytoplasm, and a relatively large, oval, hyperchromatic nucleus which frequently shows bizarre, atypical features and mitotic figures.

These classifications have presented certain difficulties: 1. The situation of a tumour involving more than one of our regional groups. In such circumstances we have endeavoured to determine the original site of origin from a knowledge of the patient's history. 2. The distinction between local recurrence and new primary growth. Here we have largely followed the principles of Sarasin (1933) and Ebenius (1943). The latter recorded as a recurrence all growths which appeared 1·0 cm. or less from the periphery of the primary lesion. 3. Variation of tumour histology according to the site of biopsy. The less differentiated group has been recorded. 4. Variation of tumour histology with the passage of time. The earliest biopsy only has been noted.

ANALYSIS OF CASES.

Table I shows the distribution of our 408 cases with regard to site and histology.

TABLE I.—*Histology and Site of the Tumours.*

Site.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.	Undiff.	Total.	Site group.	Total.	Percentage.
Lower lip	37	4	..	..	..	..	41			
Upper lip	1	..	..	..	..	..	1	1	60	15
Lip commissure	17	1	..	..	..	..	18			
Vocal cord	10	3	..	..	..	..	13			
Larynx (other)	5	..	..	..	..	..	5	2	18	4
Tongue (antr. ⅔)	59	10	..	1	1	..	71	3	71	17
Floor of mouth	31	8	1	1	..	..	41	4	41	10
Upper alveolus	5	2	..	..	..	..	7			
Lower alveolus	7	6	1	2	..	..	16			
Cheek	7	..	1	..	..	..	8	5	63	16
Palate	5	3	1	..	..	..	9			
Antr. pillar	17	3	2	1	..	..	23			
Faucial tonsil	12	17	2	6	2	..	39	6	52	13
Tongue (postr. ⅓)	5	6	1	1	..	..	13			
Postr. oropharynx	2	1	..	..	..	..	3			
Arytenoid region	10	16	5	5	4	..	40	7	54	13
Post-cricoid region	6	2	..	..	..	..	8			
Nasopharynx	..	2	..	..	1	..	3			
Nasal cavity	..	..	1	1	..	1	3			
Maxillary sinus	8	3	3	6	..	7	27	8	33	8
Ethmoidal sinus	1	1	..	..	..	1	3			
No primary found	1	4	6	1	4	..	16	9	16	4
Total	246	92	24	25	12	9	408			
Percentage	60	23	6	6	3	2				

Abbreviations employed: Kerat. = Keratinizing squamous cell carcinoma. E. kerat. = Early keratinizing squamous cell carcinoma. An. sq. = Anaplastic squamous cell carcinoma. Trans. = Transitional cell carcinoma. L-E. = Lympho-epithelioma. Undiff. = Undifferentiated carcinoma.

Fig. 1 illustrates the percentage of the different types of growth in our site groups. It will be noted that 5 of our 12 lympho-epitheliomata were located in

the pharynx (9 per cent of the group), and 2 in the tonsil (4 per cent of the group), while 4 were discovered in cervical metastases in the absence of a clinically evident primary lesion (25 per cent of the group). It is probable that the parent

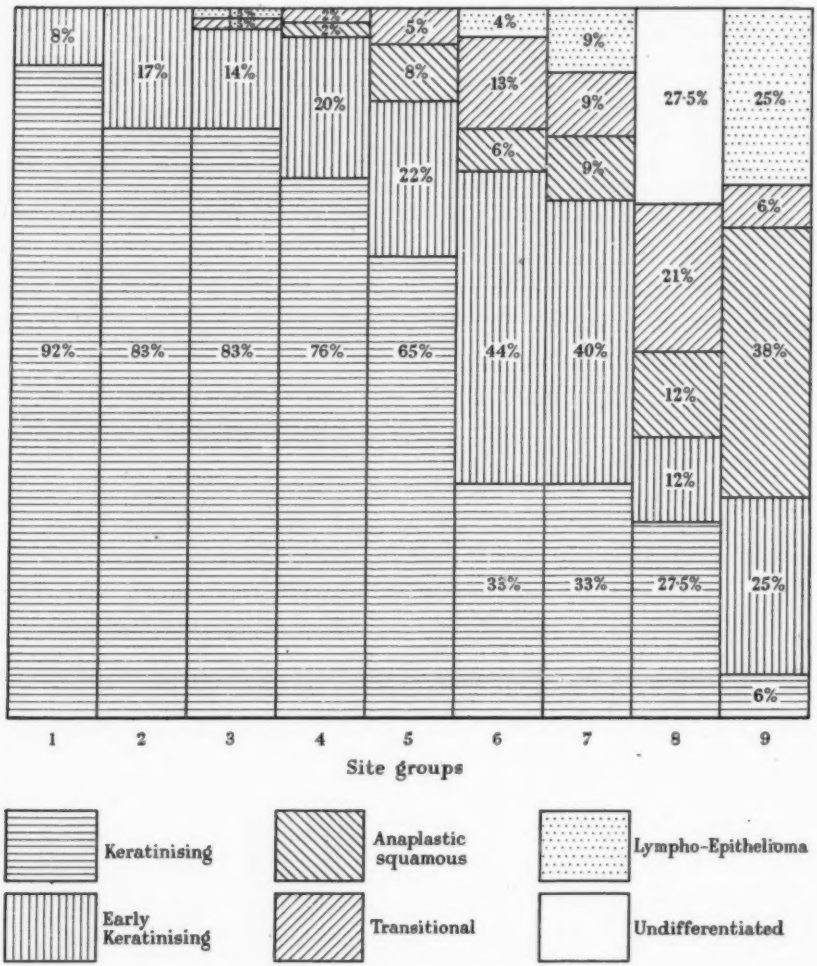

FIG. 1.—Histology and site.

growths of the latter group were in most instances pharyngeal in location. Seven of the 25 transitional cell carcinomata arose in the tonsillar region (13 per cent of the group), a further 7 in the nasal cavities (21 per cent), and 5 in the pharynx (9 per cent). Thus, with the exception of the nasal cavities, the transitional

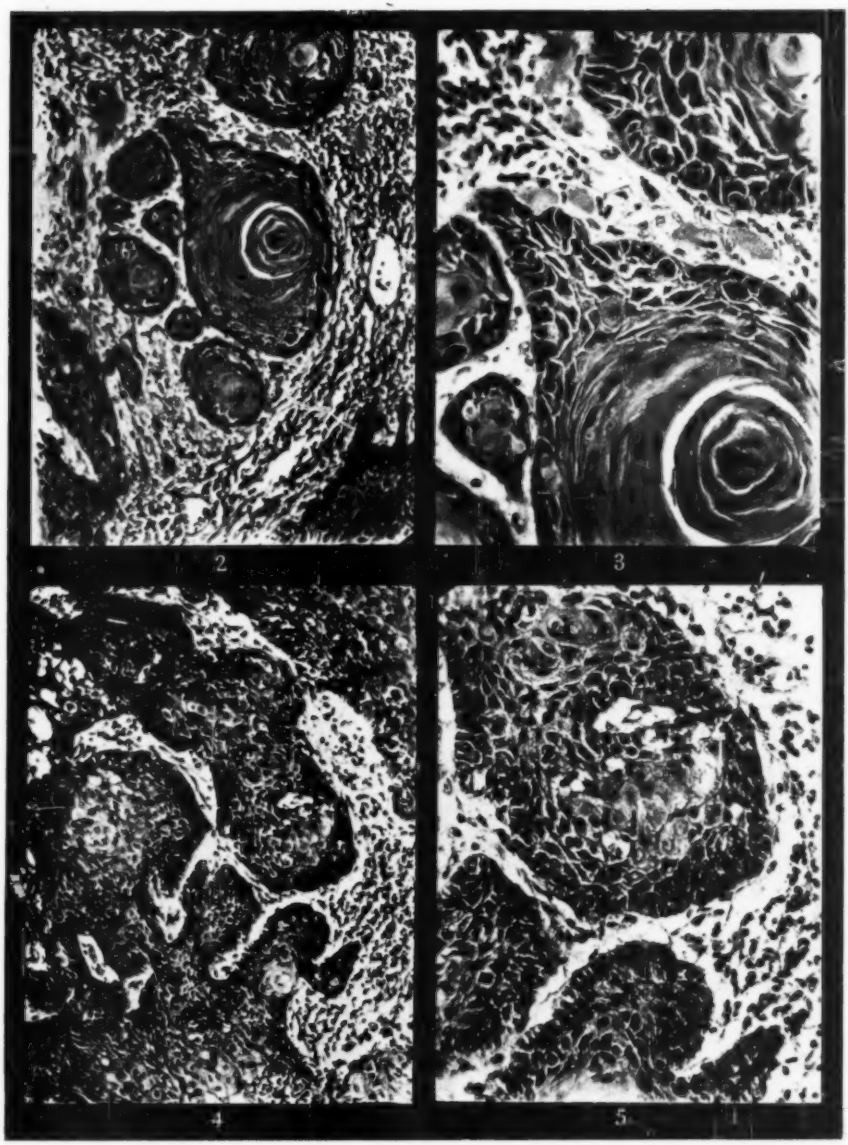

FIG. 2.—Frankly keratinizing squamous cell carcinoma. (× 100.)
FIG. 3.—Frankly keratinizing squamous cell carcinoma. (× 210.)
FIG. 4.—Early keratinizing squamous cell carcinoma. (× 100.)
FIG. 5.—Early keratinizing squamous cell carcinoma. (× 210.)

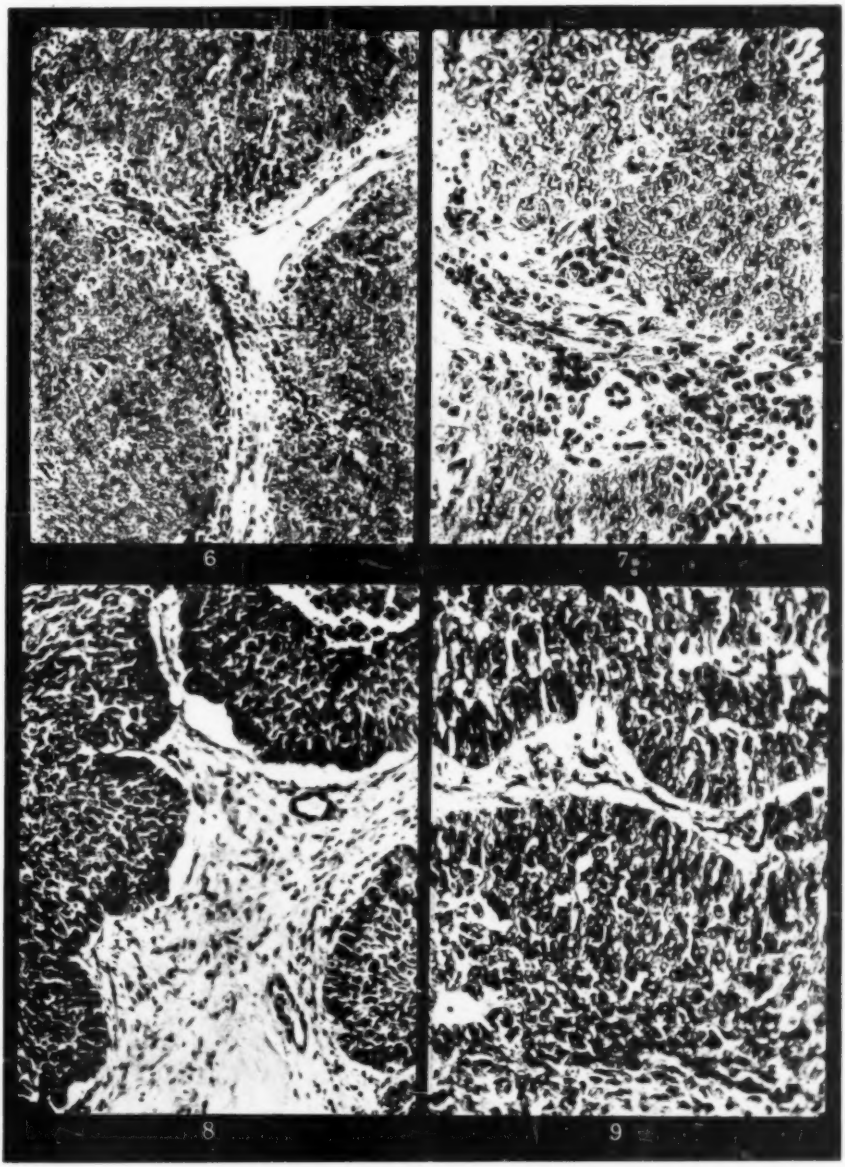

FIG. 6.—Anaplastic squamous cell carcinoma. (× 100.)
FIG. 7.—Anaplastic squamous cell carcinoma. (× 210.)
FIG. 8.—Transitional cell carcinoma. (× 100.)
FIG. 9.—Transitional cell carcinoma. (× 210.)

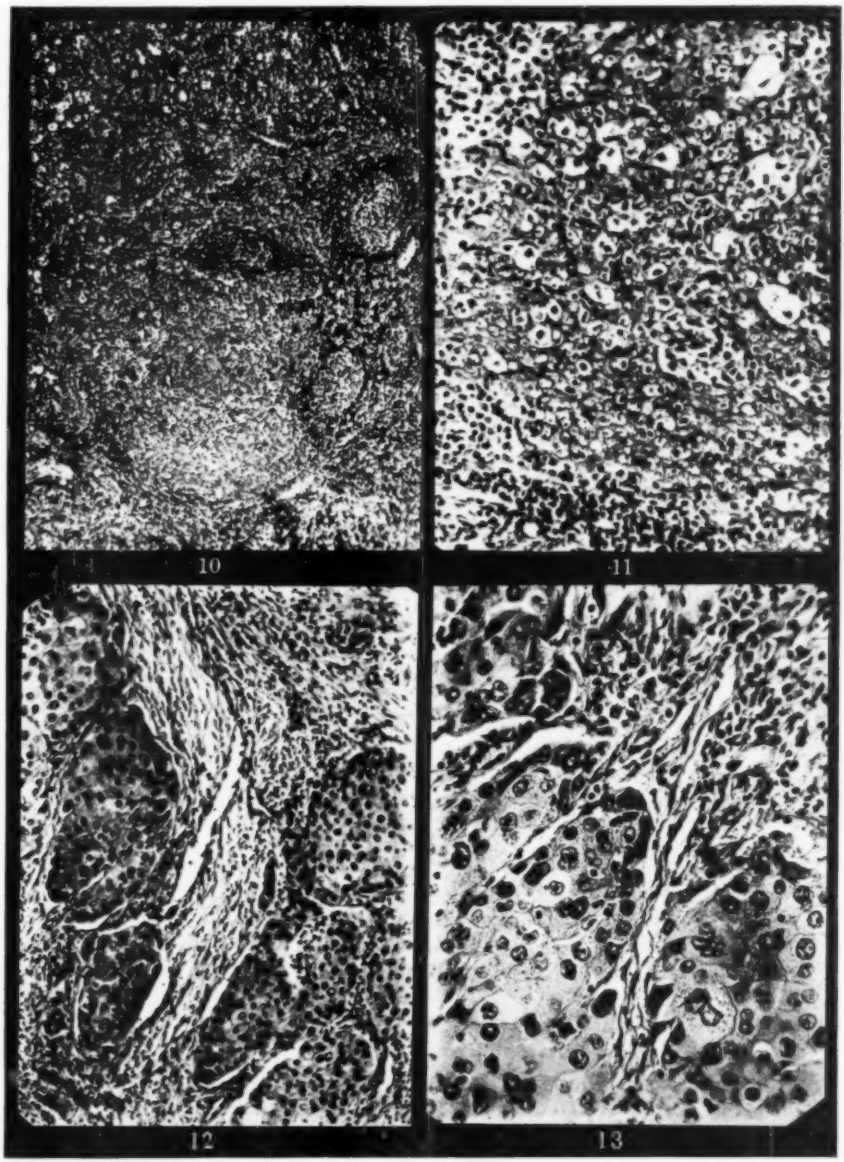

FIG. 10.—Lympho-epithelioma. (× 100.)
FIG. 11.—Lympho-epithelioma. (× 210.)
FIG. 12.—Undifferentiated carcinoma. (× 100.)
FIG. 13.—Undifferentiated carcinoma. (× 210.)

Fairburn.

cell growths seem to show the same predilection for the lympho-epithelial regions as the true lympho-epithelioma. Moreover, it will be noted that it is in such sites that the incidence of the poorly differentiated squamous cancers is highest.

Table II shows the mean ages of those suffering from cancer of various histological types. It is seen that the lympho-epitheliomata and the undifferentiated

TABLE II.—*Mean Ages of the Histological Types.*

Histology.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.	Undiff.
Mean age in years	61·5	60·5	61·0	64·5	53·5	52·5
Standard deviation	10·25	10·31	12·15	10·11	11·94	13·72

group possess the lowest mean ages (53·5 and 52·5 years respectively), while the transitional growths head the series (64·5 years). Compared with the figure for the frankly keratinizing group (61·5 years), the former two differences are statistically significant, while the latter is not. There is no significant difference between the mean ages of our cases when distributed according to the site of the primary growth. They varied from 58·5 (pharynx and nasal sinuses) to 62 years (floor of mouth and tonsil).

Table III illustrates the relationship between the histological type of the growth and the fate of the patient over a minimum period of five years. It is

TABLE III.—*Five Year Prognosis according to Histological Type.*

Histology.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.	Undiff.
Percentage dying with cancer	63·5	92·5	79·0	72·0	91.5	78.0
Median survival time in months of those dying with cancer	9·0	9·75	10·75	14·5	9·75	10·5
*Interquartile range in months	6–118	6–15	6–18	8·25–16·75	6–23	5–27·5

* If a series of 100 cases be arranged in ascending order of survival time, the range of survival time from the 25th to the 75th member of the series is the interquartile range.

seen that those possessing a frankly keratinizing tumour are the least likely to die with cancer (63·5 per cent of the group die in this manner), while, at the other extreme, 92·5 per cent and 91·5 per cent of the bearers of early keratinizing cancers and lympho-epitheliomata respectively succumb with evidence of new growth. Regarding the median survival times of those dying with cancer, that of the frankly keratinizing group is shortest (9 months), and that of transitional cell cancer is longest (14·5 months). The number of cases in this group is, however, too small to draw any definite conclusion upon a statistical basis. The remaining survivals approximate to that of the frankly keratinizing group.

Table IV shows the relationship between the histology of the primary lesion and the incidence of cervical lymph glandular extension on first examination.

TABLE IV.—*Incidence of Metastasis and Effect on Prognosis.*

Histology.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.	Undiff.
Percentage with metastasis	36	59	67	52	75	22
Percentage dying with cancer among those with metastasis	90	96	89	77	100	100
Percentage dying with cancer amongst the metastasis-free	49	87	62	67	67	71

We have adopted Ebenius' clinical criteria of metastasis. The frankly keratinizing cancers possess a statistically significant lower incidence of metastasis (36 per cent) than the remainder of their fellows, with the exception of the undifferentiated group which only metastasized in 22 per cent of cases. This table also illustrates the influence of glandular metastasis upon the likelihood of death with active growth. As expected, the presence of lymph nodal extension on first examination markedly increases the probability of death of this nature. If deposits are present there is a uniformly high incidence of death with cancer, ranging from 77 per cent in the case of transitional tumours to 100 per cent in the cases of lympho-epithelioma and the undifferentiated carcinomata. In the absence of metastasis the likelihood of a five-year cure is considerably increased in the case of the frankly keratinizing group, but with the other types of growth the improvement in prognosis is less impressive.

Table V is of a similar nature to Table III, but is concerned with the influence of tumour location. It is seen that 93 per cent of those falling into the pharyngeal

TABLE V.—*Five Year Prognosis according to Tumour Location.*

Site groups.	1	2	3	4	5	6	7	8
Percentage dying with cancer	35	55	68	73	78	90	93	82
Median survival time in months of those dying with cancer	9	23	8·5	12	11·75	9·75	7	12
Interquartile range in months	7–15	14·5–49·75	6–16	6–20	7–22·75	6–13	5–14	7–26·5

group and 90 per cent of the tonsillar group die with cancer, while at the other extreme is the lip, with an incidence of only 35 per cent. Regarding median survival time, that of the pharyngeal group is the shortest (7 months), and that of laryngeal cases the longest (23 months).

Table VI is the counterpart of Table IV. It shows the relationship between the site of the primary tumour and the incidence of glandular metastasis on first

TABLE VI.—*Incidence of Metastasis and Effect on Prognosis.*

Site Groups.	1.	2.	3.	4.	5.	6.	7.	8.
Percentage with metastasis	28	6	39	54	47	62	63	9
Percentage dying with cancer amongst those with metastasis	71	100	86	95	93	100	91	100
Percentage dying with cancer among the metastasis-free	21	53	56	47	64	80	95	81

examination. The pharyngeal and tonsillar groups possess the highest incidence (63 per cent and 62 per cent respectively), while the larynx and nasal cavities show the lowest (6 per cent and 9 per cent), and again it is seen that the presence of cervical metastases markedly increases the likelihood of death with cancer. The pharyngeal group provides an exception to this statement. The difference

between a 91 per cent incidence of death with malignancy in those with metastasis and a 95 per cent incidence in the metastasis-free is, however, insignificant. There is a uniformly high percentage of death with cancer if extension has occurred, ranging from 71 per cent in the case of the lip to 100 per cent in the cases of the larynx and nasal cavities. As in Table IV, there is considerable inter-group variation if metastases are absent, ranging from 21 per cent in the instance of the lip to 95 per cent in the case of the pharynx.

Finally, we are considering only the lympho-epithelial sites (tonsil, base of tongue, laryngopharynx (excluding the post-cricoid region), and nasopharynx).

Table VII is similar to Tables III and V. The tendency here is towards the abolition of differences between the histological groups, the incidence of death

TABLE VII.—*Five Year Prognosis according to Histological Type. Lympho-Epithelial Regions.*

Histology.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.
Percentage dying with cancer	85	100	75	75	100
Median survival time in months of those dying with cancer	8	9	9·5	11·5	20
Interquartile range in months	6–10	6–13	5·75–13	8–15	8–34·5

with cancer being 75 per cent or over in all instances. Concerning the median survival times the salient features of Table III are preserved with the exception of the longer survival of the cases of lympho-epithelioma (20 months). This is attributable to the fact that one-third of all our cases of this tumour belong to the group in which there were cervical metastases in the absence of a clinically evident primary lesion, a group which naturally carries a very low median survival time.

TABLE VIII.—*Incidence of Metastasis and Effect on Prognosis. Lympho-Epithelial Regions.*

Histology.	Kerat.	E. kerat.	An. sq.	Trans.	L-E.
Percentage with metastasis	52	68	62·5	83	71
Percentage dying with cancer among those with metastasis	100	100	80	80	100
Percentage dying with cancer among the metastasis-free	69	100	67	100	100

Table VIII should be compared with Table III. A levelling influence—tumour location—is seen at play, the range in incidence of metastasis varying only from 52 per cent for frankly keratinizing growths to 83 per cent for transitional cell cancer. It thus appears that all carcinomata arising in the lympho-epithelial regions metastasize at an early date in the clinical course of the disease whatever the histological type may be. Moreover, the presence or absence of cervical lymph glandular metastases on first examination is of little significance with regard to the likelihood of subsequent death with cancer, there being a very high incidence for all types of growth in both instances.

DISCUSSION.

Concerning the relative incidence of lympho-epithelioma in different anatomical sites, our figures bear little resemblance to those of Ch'in and Szutu (1940), who report 97 original cases. However, our figure of 4 per cent for the tonsillar group

agrees with that of Ewing (1929), who studied 200 cases. In Hoffmann's (1932) series 44 per cent of the growths were mesopharyngeal in location, 41 per cent epipharyngeal, and only 4·5 per cent hypopharyngeal. In contradistinction, 4 (33 per cent) of our cases were hypopharyngeal, in 4 (33 per cent) no primary lesion could be found, 2 (17 per cent) were mesopharyngeal, while only 1 of our 12 cases was nasopharyngeal.

Regarding transitional cell carcinoma, Quick and Cutler (1927) state that in the tonsil and base of tongue, the proportion as compared with squamous growths is 1 to 10. Our ratio is approximately 1 to 6. 65 per cent of their growths were situated in the tonsil or base of tongue, 20 per cent in the hypopharynx, and 10 per cent in the nasopharynx. (The accessory nasal sinuses were not studied.) In our series 28 per cent of these cancers belonged to the tonsil and base of tongue, 28 per cent to the nasal cavities, and 20 per cent to the hypopharynx.

The mean age of our cases of lympho-epithelioma (53·5 years) is higher than that usually reported. In Hoffmann's extensive series it was 48 years. The corresponding figure for Quick and Cutler's cases of transitional cell cancer is 52·5 years. Our figure is 65 years.

Cervical metastases were present in 88 per cent of Ch'in and Szutu's cases of lympho-epithelioma. Our figure is 75 per cent. Frank, Lev and Blahd (1941), in their study of transitional cell growths, report a 44 per cent incidence of metastasis. 52 per cent of our cases possessed evidence of glandular extension on first examination.

Inspection of our data discloses the relative insignificance of tumour histology as a prognostic factor in cases of cancer of the lympho-epithelial sites. MacCarty (1922) and Plaut (1927) have emphasized its limitations, stressing the far greater importance, in most instances, of clinical features, such as the age and general condition of the patient, the duration, location, and gross anatomy of the growth, and the presence, or absence, of metastasis. The enormous preponderance of the site factor over that of histology is borne out by this study. Thus, frankly keratinizing neoplasms of the lip carry a relatively good prognosis, but when arising in a lympho-epithelial region their significance is as sinister as that of their ill-differentiated fellows. The presence of lymph-node metastasis on first examination is, in our experience, a factor of far greater prognostic value than tumour histology, although very appreciably influenced by the site of the parent growth and its accessibility to treatment. To take a specific example, metastasis in a case of lip cancer markedly decreases the likelihood of cure, whereas when a lympho-epithelial region is involved, death with active malignancy is likely to occur whether the cervical nodes are involved or not.

The chance of invasion of the cervical lymph glands appears to be largely independent of the histological grouping, the site of origin of the tumour being of infinitely greater significance. It has been widely assumed that the main factor in dissemination is the inherent growth capacity of the neoplasm. But this is not the only influence which has to be considered. The regional extension of carcinoma has been shown conclusively to be by way of lymphatics. Therefore, *a priori*, there must be a greater tendency to early metastasis in tumours arising in those areas which possess a rich lymphatic plexus—a fact which appears to have been insufficiently considered. The ill-differentiated buccopharyngeal carcinomata have gained an evil reputation for early and widespread metastasis, a phenomenon which is usually attributed to rapidity

of growth or prolonged clinical latency. We believe that this character is due in the main to the predilection of these tumours for such sites.

SUMMARY.

408 cases of buccopharyngeal carcinoma have been followed to death or for a minimum period of five years.

An attempt has been made to elucidate further the natural history of the less differentiated types occurring in this region.

It is concluded that the location of such tumours is of far greater prognostic significance than their histological appearance.

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SARCOMA AND MELANOMA OF THE RECTUM.

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SARCOMA and melanoma are relatively rare forms of malignant disease in the rectum. At St. Mark's Hospital we have met with only eight cases of sarcoma and two of melanoma during the last 18 years (1929 to 1946 inclusive), whereas during this period we examined more than 2,200 operation specimens of rectal carcinoma. Judged from this experience sarcoma and melanoma constitute approximately 0·5 per cent of malignant rectal tumours. This agrees fairly closely with the estimates of other observers (Weeks, 1927; Sutton, 1932; Kallet and Saltzstein, 1932; McSwain and Beal, 1944).

As we met with these tumours one by one at infrequent intervals we noticed that they varied considerably in their histology, but now that we have collected and reviewed a small series of cases it is clear that they fall naturally into four groups, namely lymphosarcoma, reticulum cell sarcoma, spindle cell sarcoma and melanoma. It will save repetition if before describing our cases we mention the chief points in the histology of each of these groups.

HISTOLOGY OF TUMOURS.

Lymphosarcomas of the rectum are malignant tumours composed of round cells resembling, though slightly larger than, the lymphocytes of the blood. The nuclei are round, stain deeply and are surrounded by a narrow ring of cytoplasm (Fig. 1). Both large "lymphoblastic" and small "lymphocytic" cells may be represented in the same tumour. (We prefer the familiar term lymphosarcoma to the alternative names which have been suggested in recent years, such as lymphocytoma, lymphocytic reticulo-sarcoma or lymphoid-reticulocytoma). Four of our ten cases were lymphosarcoma, one being associated with an adenocarcinoma.

The chief distinguishing feature of a *reticulum cell sarcoma* is the presence of a well developed intercellular argyrophile reticulum, but apart from this the individual tumour cells are much more variable in size and shape than in a lymphosarcoma (Fig. 2). Polygonal and pear-shaped cells with spur-like projections of acidophil cytoplasm are generally numerous and these have large nuclei which stain deeply and often show a prominent nucleolus. Reticulum cell sarcoma is not so commonly met with as lymphosarcoma, and only two of our cases belong to this group.

Spindle cell sarcomas are distinguished from the preceding two tumours by the fact that the tumour cells are elongated, slender and pointed. The nuclei are also elongated and have blunt or rounded ends (Fig. 3). Two of our cases had this histology and we regarded them as leiomyosarcomas derived from the unstriped muscle of the rectum.

The distinctive characteristic of *melanoma* is of course the presence of melanin pigment (Fig. 4). This pigment is irregularly distributed in rectal melanoma,

and in our two cases was more obvious in the glandular metastases than in the primary tumour. In one of our cases of melanoma an adenocarcinoma was also present in the rectum.

DETAILS OF CASES.

1. *Lymphosarcoma.*

Case 1.—E. O—, female, aged 37. Sigmoidoscopic examination revealed a round tumour in the posterior quadrant of the lower third of the rectum which was biopsied and reported "lymphosarcoma." 15.i.1942: Perineo-abdominal excision by Mr. W. B. Gabriel. The operation specimen showed a round protuberant tumour mostly covered by intact mucosa, but ulcerated in the lower half at the site of the previous biopsy (Fig. 5). The histology was typical of lymphosarcoma, the cells being chiefly of the "lymphoblastic" type. Local spread had occurred upwards in the submucosa for a short distance, and there was infiltration of adjacent rectal muscle but no noticeable spread in perirectal fat. No growth found in veins, but metastases were present in twelve out of 27 glands examined. The position of these glandular metastases is shown in the accompanying photograph of the gland dissection (Fig. 6). After-history—no sign of recurrence five years after operation and general health is now good.

Case 2.—Mrs. B—, aged 57. Abdominoperineal excision of rectum by Mr. Lawrence Abel, 9.ii.1943. Operation specimen showed an ulcerated tumour 2½ inches in diameter encircling rectal ampulla. The tumour was softer in consistence than most carcinomas and the base of the ulcer was covered by a greyish slough. Histology typical of lymphosarcoma. Both "lymphoblastic" and "lymphocytic" cells present. Extensive spread in rectal muscle and commencing invasion of perirectal fat. No growth in veins and no lymphatic metastases (Fig. 7). After-history: In good health four years after operation.

Case 3.—J. H—, male, aged 60. Excision of rectum by Mr. E. T. C. Milligan, 31.viii.1943, for large tumour seen through sigmoidoscope in lower third of rectum. Examination of operation specimen revealed the presence of two other unsuspected tumours in recto-sigmoid region and also a diffuse infiltration of the submucosa in lower part of pelvic colon (Fig. 8). All these tumours showed the typical histology of lymphosarcoma. The tumour in the lower third of the rectum had spread extensively in the submucosa, but only slightly in the rectal muscle. No growth was found in veins and all the lymphatic glands were free from metastases. After-history: The patient was alive and well three years and four months later.

Case 4.—D. J—, male, aged 63. Tumour first discovered in ano-rectal region in August, 1946. Biopsy taken and variously reported by different pathologists but all agreed on malignancy. Patient's general health good. Blood count normal. No significant facts in past history other than operation five years previously in Jamaica "for piles, fistula and fissure." Tumour accepted as malignant and abdominoperineal excision of the rectum carried out by Mr. Donald Barlow, 11.xi.46.

Examination of operation specimen revealed what seemed to be one single round protuberant growth 3 inches in diameter, almost completely encircling the lower third of the rectum and extending down into the anal canal (Fig. 9). The tumour was dark purple in colour and fleshy in consistence. The centre was ulcerated, but the raised margins were covered with intact mucous membrane.

Microscopic examination unexpectedly disclosed two different types of neoplasm. The main bulk of the tumour had the histology of a lymphosarcoma (lymphoblastic type), but the lower and posterior half appeared to be a well-differentiated adenocarcinoma (Fig. 10). The boundary between the two tumours was sharp and distinct. The lymphosarcomatous tumour had spread in the mucous membrane and submucosa and only to a slight extent in the rectal muscle. The adenocarcinoma had infiltrated more deeply, but there was no sign of venous spread and the lymphatic glands were all free from metastases.

We have given careful consideration to the possibility that this might actually be only one tumour, exhibiting an undifferentiated pattern in one part and a differentiated glandular pattern in another, as was suggested by Dr. W. F. Harvey, who very kindly examined the sections for melanin pigment. We are deeply indebted to Dr. Harvey for his willing help and valuable advice and agree with him that rectal tumours often differ in their histology in different regions. But when this occurs regions can be found where the histology is intermediate in character and one part runs into another. In this tumour there was no such intermediate zone. One part consisted of a soft bulky superficial tumour spreading in the submucosa, uniform in its histology in all parts and resembling in appearance a lymphosarcoma. The other part was a typical adenocarcinoma, well differentiated in character and judged from its histology not of a high grade of malignancy. The border line between the two tumours was sharp and distinct, and after giving careful consideration to all other possibilities we conclude that the most probable explanation is that a lymphosarcoma and an adenocarcinoma developed separately from points in close proximity and became fused to form one tumour.

DESCRIPTION OF PLATES.

PLATE I.

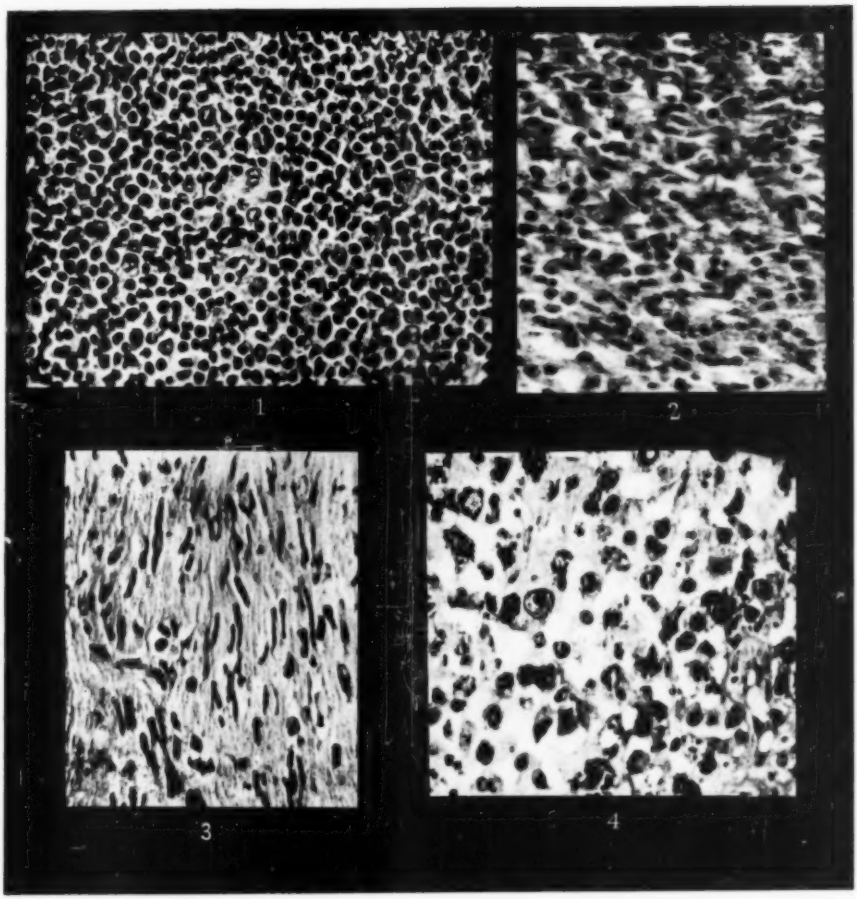
FIG. 1.—Lymphosarcoma of rectum. × 500. (Case 1.)
FIG. 2.—Reticulum cell sarcoma of rectum. ×500. (Case 5.)
FIG. 3.—Spindle cell sarcoma of rectum. × 500. (Case 8.)
FIG. 4.—Melanoma of rectum. × 500. (Case 9.)

PLATE II.

FIG. 5.—Surface view of lymphosarcoma of rectum. (Case 1.)
FIG. 6.—Gland dissection of lymphosarcoma showing position of metastases. (Case 1.)
FIG. 7.—Surface view and gland dissection of lymphosarcoma of rectum. (Case 2.)
FIG. 8.—Surface view of lymphosarcoma of rectum. Multiple tumours marked by arrows. (Case 3.)
FIG. 9.—Surface view of combined lymphosarcoma and adenocarcinoma of rectum. (Case 4.)
FIG. 10.—Sections through tumour in Case 4. (A) Region showing lymphosarcoma. × 100. (B) Region showing adenocarcinoma.

PLATE III.

FIG. 11.—(A) Surface view of operation specimen of reticulum cell sarcoma showing multiple tumours. (B) Gland dissection to show position of metastases. (Case 5.)
FIG. 12.—Surface view of pedunculated spindle cell sarcoma of upper third of rectum. (Case 8.)
FIG. 13.—Surface view of operation specimen and photograph of gland dissection in case of double malignancy. Tumour B is a melanoma with four lymphatic metastases containing melanin. Tumour A is an adenocarcinoma with two lymphatic metastases free from pigment. (Case 9.)
FIG. 14.—Double malignancy in rectum. Section of Tumour A showing adenocarcinoma, and of B showing melanoma (both × 160). (Case 9.)



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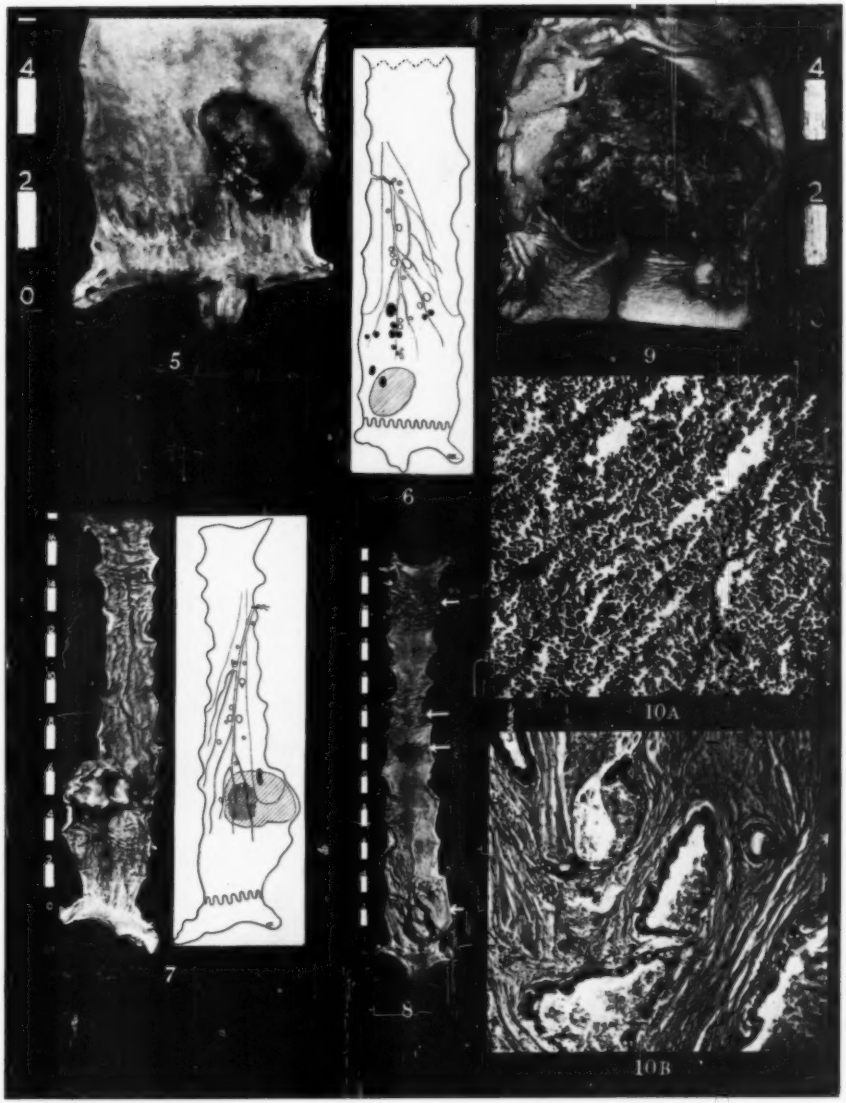
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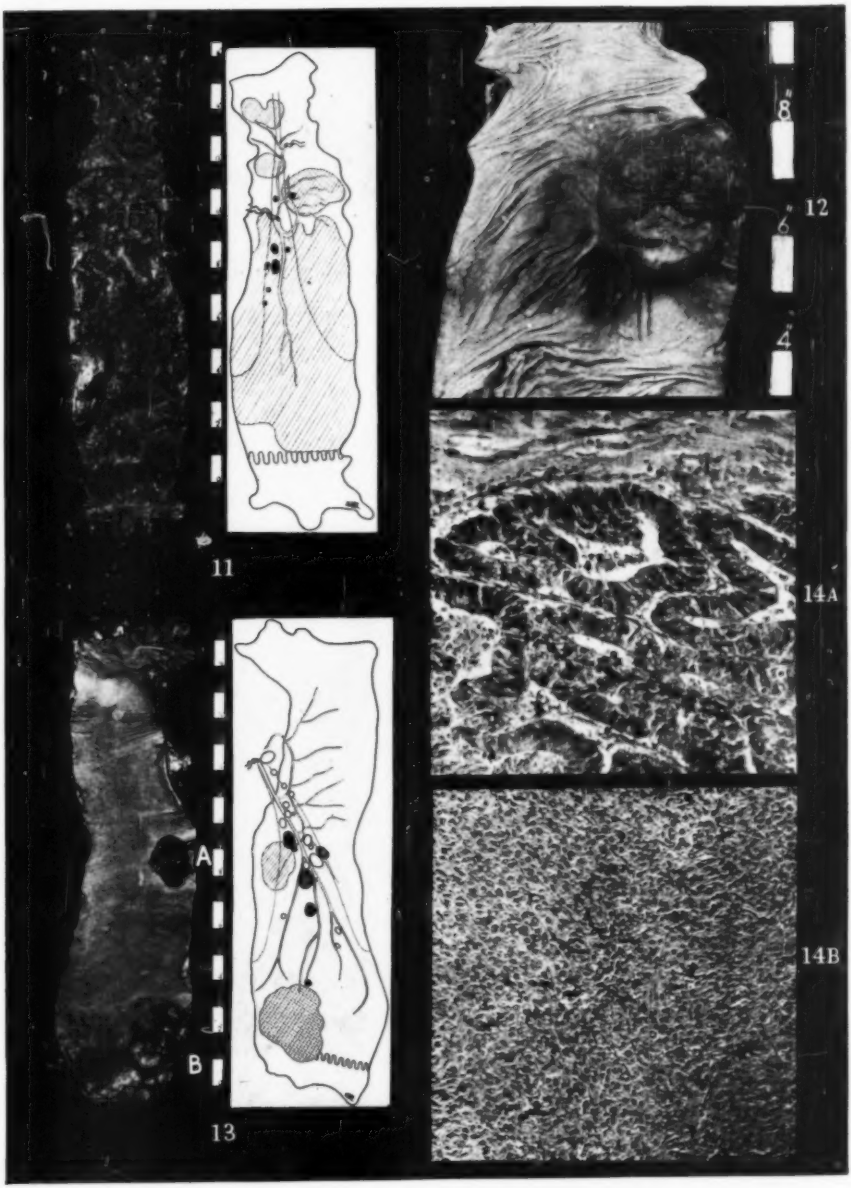
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2. *Reticulum cell sarcoma.*

Case 5.—Mrs. G—, aged 61. Perineo-abdominal excision by Mr. W. B. Gabriel, 17.i.1940, for extensive malignant disease in rectum. Operation specimen showed widespread ulceration in the rectum, recto-sigmoid region and distal end of pelvic colon, accompanied by very many black spongy projecting tumours (Fig. 11*a*). Histology typical of reticulum cell sarcoma. Diffuse spread resulting in haemorrhoidal vessels being embedded in local deposits. Eight lymphatic metastases. Position of these glandular metastases shown in photograph of gland dissection (Fig. 11*b*). After history: Patient died from cardiac failure the day after operation.

Case 6.—H. B—, male, aged 45. Found by Mr. W. B. Gabriel to have huge nodular tumour in lower third of rectum which prolapsed from anus. Biopsy showed sarcoma of reticulum cell type. Case considered inoperable because of extensive spread. Glandular swellings developed later and patient died within five months. No autopsy.

3. *Spindle cell sarcoma.*

Case 7.—J. H—, male, aged 60. First attended St. Mark's Hospital, 17.i.1945, and found to have a fibrous nodular tumour on posterior quadrant of lower third of rectum. Biopsy showed spindle cell sarcoma apparently of low grade malignancy. Seven years previously patient had had the coccyx removed in another hospital, and three years later a small tumour had been found in the rectum which was reported as a mass of fibrous tissue. Synchronous combined excision of rectum by Mr. O. V. Lloyd-Davies, 21.iii.1945. Operation specimen showed a small nodular tumour with smooth rounded margin situated in lower third of rectum. Histology typical of leiomyosarcoma. Slight spread into perirectal fat. No venous spread and no lymphatic metastases but a hard nodule in perirectal fat one and a half inches above the primary tumour proved to be a secondary deposit. After history: In good health 20 months after operation.

Case 8.—I. W—, male, aged 70. Found to have large dark pedunculated tumour in upper third of rectum. Biopsy showed sarcoma, composed of spindle cells. Severe haemorrhage after biopsy. Perineo-abdominal excision of rectum by Mr. W. B. Gabriel, 19.x.1946. Operation specimen showed huge round tumour situated on left lateral and anterior quadrants of upper third of rectum, firm in consistence, dark purple in colour and ulcerated on surface (Fig. 12). Histology—spindle cell sarcoma, probably leiomyosarcoma. Evidence of spread through rectal muscle and commencing extension to perirectal fat. No sign of venous spread or lymphatic metastases. After history: Death from renal failure six days after operation.

4. *Melanoma.*

Case 9.—R. R—, male, aged 56. First seen by Mr. Nevin, 14.ii.1939, who found a large tumour in lower third of rectum. Biopsy report—melanoma. Abdomino-perineal excision of rectum March, 1939. Examination of operation specimen revealed two tumours, one in lower and one in upper third of rectum (Fig. 13).

The lower tumour (B) was a dark protuberant growth which had spread into the muscles of the ano-rectal ring. Histology typical of melanoma. Distri-

bution of melanin pigment very irregular, some areas being almost free, others containing large quantities. Metastases present in four out of 21 glands. Metastases heavily pigmented with melanin ; position shown in photograph of gland dissection.

Tumour in upper third of rectum (A) was an oval ulcerated growth 2 inches in diameter. This was a typical adenocarcinoma, well differentiated in character, and judged from its histology of a low grade of malignancy (Fig. 14). Two regional lymphatic metastases from this. More than 4 inches of healthy bowel separated the melanoma from the adenocarcinoma. Both tumours had given rise to lymphatic metastases, those from the melanoma being pigmented, whereas those from the adenocarcinoma contained no melanin. A unique pathological specimen. After history : Death from recurrence after one year and three months.

Case 10.—V. M—, male, aged 53. At first visit to hospital, 28.i.46, Mr. W. B. Gabriel found a tumour in the ano-rectal region which prolapsed from the anus. Biopsy revealed melanoma. Massive submucous infiltration developed in a few days and condition considered to be inoperable. Death from generalized disease within nine months.

DISCUSSION.

The number of cases of sarcoma and melanoma of the rectum recorded in the literature is not large. Twenty years ago Weeks (1927) found reference to 100 cases of rectal sarcoma, but he regarded many as of doubtful validity and remarked—" If the questionable cases were discarded it is certain that the number would be reduced by one half." We agree with this comment and have formed the impression that many of the cases which until recent years were recorded in the literature as " sarcomas " were probably examples of anaplastic carcinoma. This criticism does not apply to the cases reported in the last 15 to 20 years.

Most authors who have written about this subject seem to take the view that all varieties of sarcoma tend to run a rapidly fatal course, but we think that this attitude needs qualification. Each of the four varieties of malignancy has to some extent its own characteristics and peculiarities and the prognosis differs considerably in each.

Lymphosarcoma is the commonest form of sarcoma of the rectum. It may manifest itself either as a single localized tumour or give rise to a crop of tumours spread over a long stretch of the bowel. Bensaude (1929) was one of the first to draw attention to the diffuse or generalized variety of lymphosarcoma. In one of our cases (Case 3) the disease affected the whole rectum and distal end of the pelvic colon, so that in describing the primary focus we could not speak of a particular site of origin, but only of a region of involvement. The largest tumour was in the lower third of the rectum, but the other smaller tumours at a higher level were certainly not secondary growths resulting from the transportation of tumour cells, but were obviously new foci of lymphosarcoma developing independently. In spite of this unfavourable feature of lymphosarcoma the prognosis after surgical treatment would seem to be fairly good on the whole. Our four patients with this disease are all alive and well, one five years, one four, and one three years and four months after rectal excision. The fourth case is a very recent one, but this patient also is in good health, though it is only three months since the operation. Good results in lymphosarcoma have also been reported

after radiotherapy and Cutler (1935) says that even the generalized form of the disease may respond to roentgen therapy.

In its gross characters reticulum cell sarcoma is indistinguishable from lymphosarcoma and this too may manifest itself in either a localized or generalized form. When further cases have been kept under observation it will be interesting to notice in what respects, if any, the course of this form of malignancy differs from that of lymphosarcoma. At present there is insufficient evidence on which to base a comparison because it is only within recent years that these tumours have been separated out from other varieties of sarcoma. Interest in the subject of reticulosis, stimulated by the writings of Robb-Smith (1938), has had considerable influence in this respect. Reticulum cell sarcoma is certainly a rarer tumour in the rectum than lymphosarcoma, and we think that it will probably be found to have a worse prognosis.

Spindle cell sarcoma is also a rare form of malignancy in the rectum. It is the one most easy to distinguish clinically from carcinoma because it obviously arises from the tissues of the bowel wall and not from the surface mucous membrane. The firm solid structure and covering of intact mucosa are of special significance. It has already been mentioned that in their histology these tumours give the impression of being of a relatively benign character.

All who have written about melanoma speak of it as the most malignant of all the tumours now being considered. We agree with the view expressed by Linder and Wood (1936) that melanomas arise from the skin of the anal canal and tend to grow up into the rectum and that they metastasize early. The lymphatic glands may contain more pigment than the primary tumour, but it is worth mentioning that the haemorrhoidal and paracolic lymphatic glands may contain melanin apart altogether from the presence of a melanotic tumour. We have often found phagocytic cells loaded with melanin in the sinuses of lymphatic glands in cases of melanosis coli or pigmented colon. The pigment may be sufficient to make the whole gland dark brown in colour. On the other hand it must be recalled that in some melanomas the pigment may be very scanty. Kallet and Saltzstein (1932) have commented on this and Galard Paris and Roca de Vinals (1946) have reported a rectal melanoma with no pigment at all.

TABLE I.—*Points of Distinction between Carcinoma, Sarcoma and Melanoma.*

	Carcinoma.	Sarcoma.	Melanoma.
Site	Anywhere in rectum	Anywhere and everywhere !	Always in ano-rectal region.
Appearance	Most commonly an oval ulcerated tumour with raised margins	At first a protuberant growth with intact mucosa. Ulcerated later	Dark tumour resembling thrombosed haemorrhoids.
Consistence	Feels solid and hard	Rather soft ; resembles brain tissue after fixation	Firm, but not hard.
Cut surface (after formalin fixation)	Yellowish-white	Greyish-white	Light to dark brown.

Although the cases we have examined personally are only ten in number they do provide material for comparison with the much commoner tumour, carcinoma of the rectum. In the end, of course, these rarer forms of malignancy can only be identified by detailed study of their histology, but there are some features in their gross characters which would make an experienced surgeon suspect that he was dealing with something "out of the ordinary." We have summarized these by setting them out in tabular form (Table I).

Finally we have left to the end what we consider to be the most interesting feature of these rare tumours, namely, the frequency of multiple foci of malignancy. In four of our ten cases more than one tumour was found in the rectum, and in two cases the second tumour was of a completely different histological type. This raises the question both of multiple malignancy and dissimilar primary tumours.

Multiple malignancy is commoner in the rectum than is usually supposed. It so happens that we are in a position to give facts about this question because for many years we have been making observations on the frequency of the occurrence of multiple primary carcinoma and have found that in operation specimens of rectal cancer removed by a combined operation more than one primary rectal carcinoma is present in 4 per cent of cases (over 2,000 examined). Moreover we have records of many additional cases in which a second carcinoma was present in the colon in association with rectal cancer, so we were not surprised to find multiple tumours in our cases of lymphosarcomas; in fact, we expected this to be the case.

The occurrence of dissimilar primary tumours was, however, a complete surprise, and as far as we have been able to find out has not been previously recorded as a characteristic of these rarer forms of malignancy. In any organ of the body the existence of two tumours of a different histological type is a rare phenomenon. It is indeed remarkable that this unusual combination should have occurred twice in a series of only ten cases. We have searched through the literature but found no reference to the simultaneous occurrence of carcinoma and sarcoma in the rectum, though Kreibig (1929) mentions a case of carcinoma plus lymphosarcoma in the ileo-caecal region. As far as the general relationship between carcinoma and sarcoma is concerned we note that Warren and Gates (1932) in a study of this subject were only able to collect from the literature 25 instances of the co-occurrence of carcinoma and sarcoma as separate tumours in the same organ. Three were cases of sarcoma and carcinoma of the breast, twelve were of the uterus, and ten were in miscellaneous organs, including four cases of the combination of carcinoma and lymphosarcoma of the stomach. They found no recorded case of carcinoma and sarcoma of the rectum or indeed of carcinoma of the rectum associated with sarcoma of any other part of the intestine. We regarded our first case (No. 9) as an accidental coincidence, but the discovery of a second case (No. 4) in such a small series as ten cases seems evidence that the association is greater than could be attributed to chance. We have no explanation of this strange phenomenon, but put it on record in case it may help to supply some missing bit to the jig-saw puzzle of the origin of malignant disease.

CONCLUSIONS AND SUMMARY.

Both sarcoma and melanoma of the rectum are rare forms of malignancy constituting together approximately 0·5 per cent of malignant rectal tumours.

They cannot always be distinguished from carcinoma by clinical examination, but often they exhibit some peculiar feature which makes an experienced surgeon suspect that he is dealing with an unusual neoplasm. They are comparatively easily identified by biopsy. According to their histology these tumours fall naturally into four groups: (1) Lymphosarcoma; (2) Reticulum cell sarcoma; (3) Spindle cell sarcoma, and (4) Melanoma. Lymphosarcoma is the commonest of these tumours. It may manifest itself as a single tumour or in a diffuse or generalized form. In our cases excision of the rectum seems to have been successful treatment, and it is probable that patients with lymphosarcoma of the rectum who are treated by a combined excision operation have a relatively good prognosis. Reticulum cell sarcoma may also assume a diffuse or generalized form. It is a rarer tumour than lymphosarcoma and probably has a worse prognosis. Spindle cell sarcoma is also a very rare tumour, but appears to be of a relatively benign character. Melanoma arises always from the ano-rectal region and spreads upwards into the rectum. It metastasizes early and runs a rapid course.

In four of the ten cases we are reporting more than one malignant tumour was present in the rectum. One of these was an example of multiple lymphosarcoma and another of multiple reticulum cell sarcoma. In these cases the associated tumours were of the same histological type, but in two other cases the associated tumours were of completely different histological type. One of these was an example of melanoma associated with adenocarcinoma, and the other of lymphosarcoma associated with adenocarcinoma. We conclude from this experience that a general tendency towards multiple foci of malignancy is a distinguishing characteristic of sarcoma and melanoma of the rectum.

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ABNORMAL MITOSIS IN TUMOURS.

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It has frequently been noted by the early investigators of tumour pathology that besides a high mitotic rate, tumour cells may also show great variation in the details of mitosis (Pianese, 1896; Hansemann, 1904). A high frequency of abnormal division may indeed be an almost universal phenomenon in tumours (Ludford, 1942). The abnormalities may concern the behaviour of the chromosomes or the cytoplasm or both, and most of them lead to degeneration and death of the cells. This is largely due to the deficient nuclei which usually result from abnormal mitosis. The great wealth of evidence obtained in radiation experiments indicates that normal cellular activity stops when the nucleus does not contain the full chromosome complement (Koller, 1943*a*). It is obvious that cells which are undergoing abnormal mitosis contribute little to the growth of tumours, but they may have some other role of importance in respect of the histological organization of the tumour. It needs to be emphasized that the growth rate is determined primarily by the number of normally dividing cells.

Recent cytological studies on a great number of human tumours of various kinds show that abnormal cell behaviour is generally due to external conditions (e.g. reduced food supply). Evidence has also been obtained, however, which shows that abnormalities of tumour mitosis can also be conditioned from within the cell.

The present paper describes various cytological types of abnormal cells which are commonly encountered in tumours, and analyses the causes underlying them. In view of the fact that abnormalities in dividing tumour cells are also induced by various therapeutic agencies (e.g. X-rays, γ-rays, and chemical means), it seems desirable to describe and illustrate those mitotic irregularities which occur in tumours naturally.

EXTERNALLY CONDITIONED ABNORMALITIES.

The cell population of infiltrating tumour cords is stratified in various cell types, which can readily be distinguished by the morphological characteristics of their nuclei. In many investigations it has been seen that dividing cells are most numerous at the periphery of the tumour mass and in regions around blood-vessels. Mitotic abnormalities are rare in cells of these regions (Fig. 1). Towards the centre of the mass, however, while the number of dividing cells decreases, the frequency of cells showing mitotic abnormalities increases (Table I). The cytological differentiation of tumour tissue into various cell types with different functions, indicated by their different morphology and nucleic acid content, is correlated with the food supply (Caspersson and Santesson, 1942).

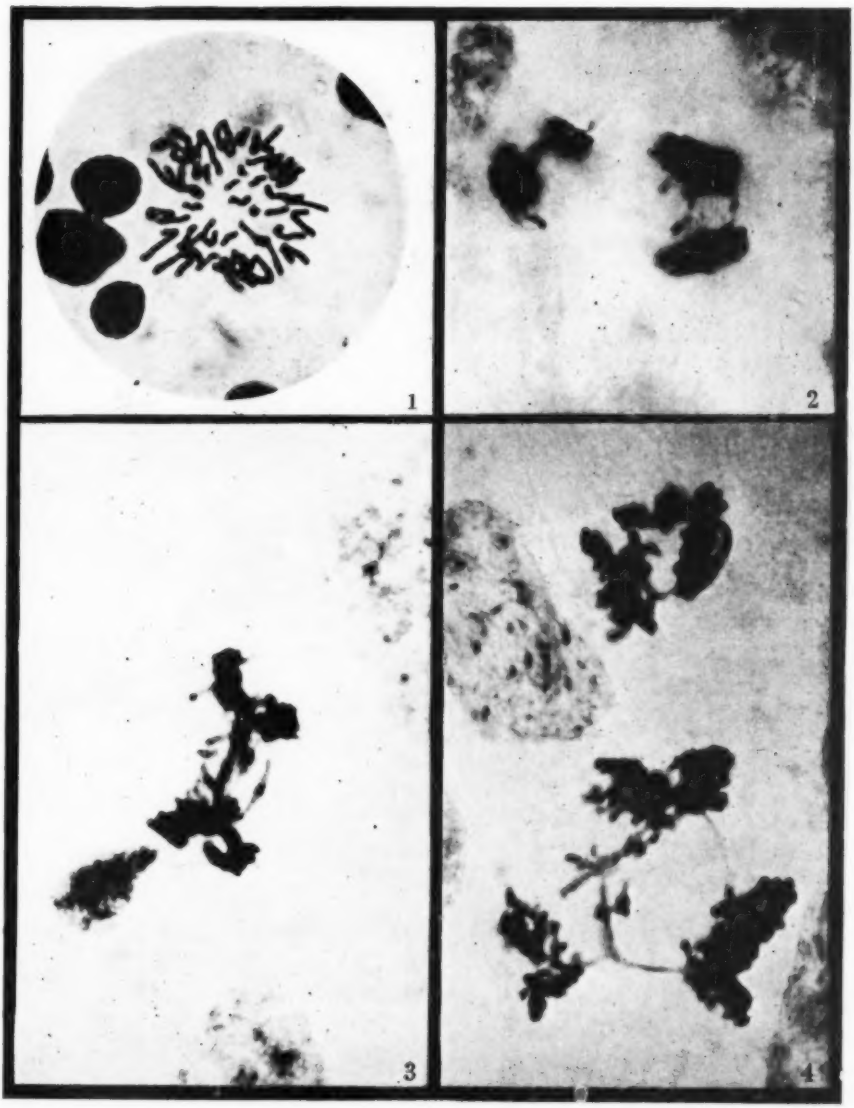

FIG. 1.—Tumour cell in metaphase showing the normal 48 chromosomes (carc. cervix). × 2,500.
FIG. 2.—Two tumour cells in anaphase with chromosome bridges and lagging chromosomes (carc. lip). × 2,000.
FIG. 3.—Telophase showing extreme stickiness of chromosomes, which prevents their separation (carc. cervix). × 2,000.
FIG. 4.—Owing to chromosome bridges, tumour cells remain in association during following mitosis (carc. breast). × 2,000.

Koller.

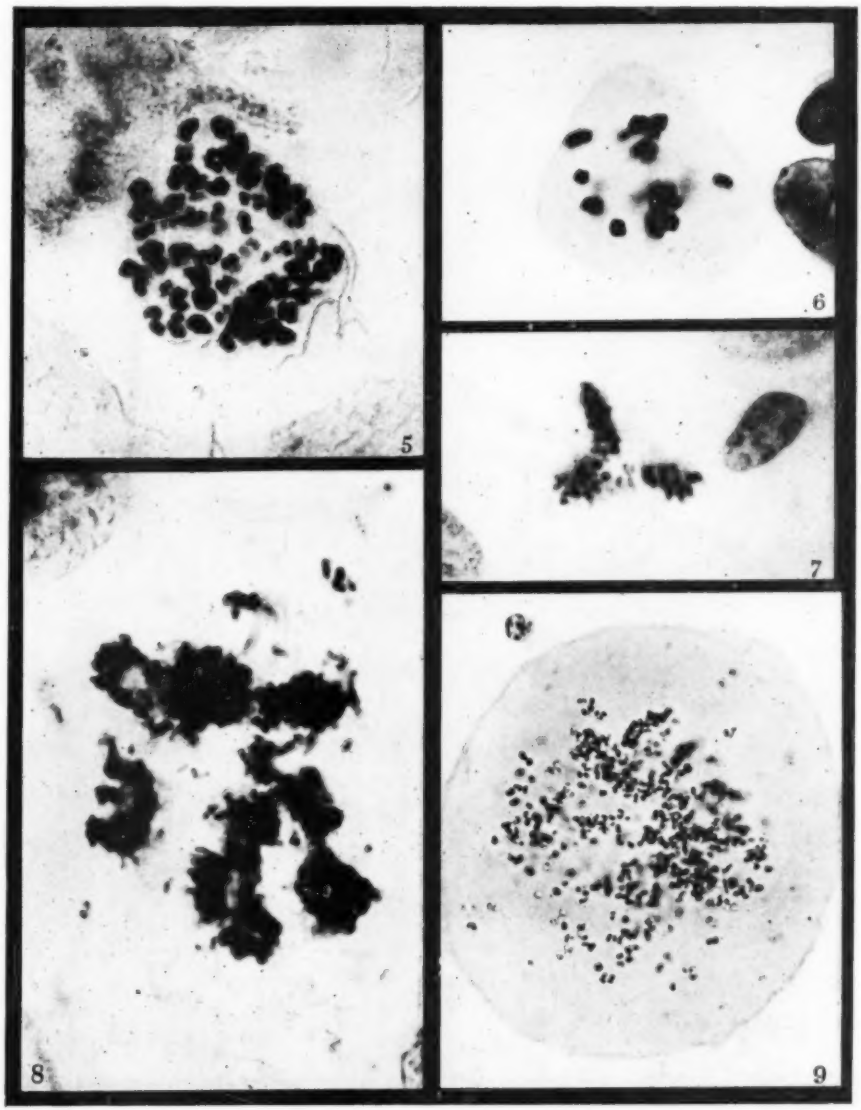

FIG. 5.—Over-spiralization of metaphase chromosomes due to suppression of spindle formation (carc. cervix). × 2,000.

FIG. 6.—Over-condensation of metaphase chromosomes. Only 16 chromosomes are present instead of 48 (carc. rectum). × 2,000.

FIG. 7.—Metaphase with tri-polar spindle (carc. cheek). × 1,800.

FIG. 8.—Synchronous division in a multi-nucleate cell (carc. cervix). × 1,800.

FIG. 9.—Free giant tumour cell with about 250 small chromosomes. (Aspirated material from abdomen in a case of carc. of alimentary canal.) By permission of Mr. L. La Cour. × 1,000.

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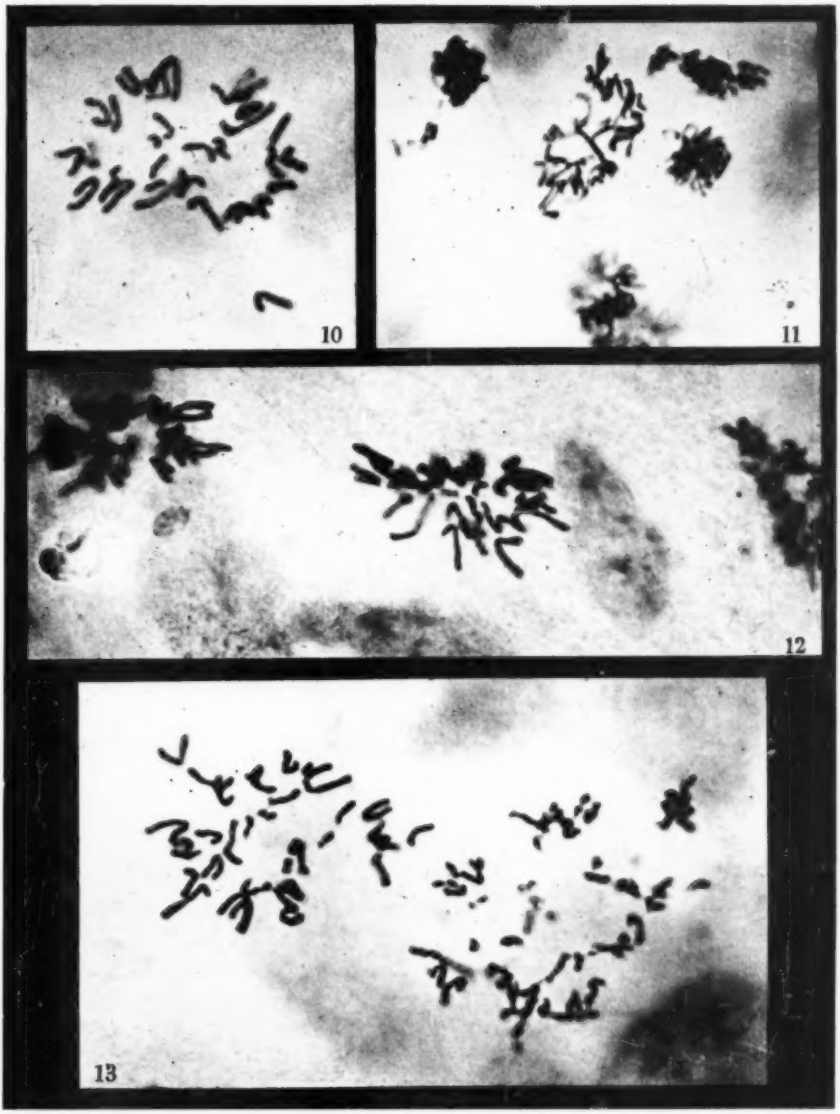
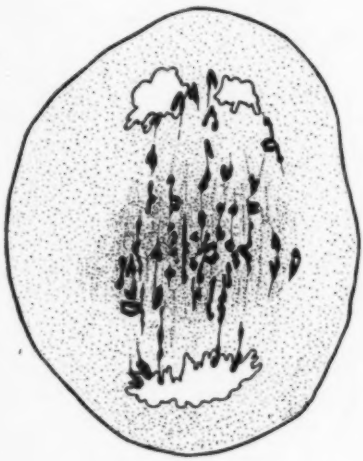


FIG. 10.—Metaphase with 32 chromosomes (carc. rectum). × 2,000.
FIG. 11.—Adjacent cells having only 24 chromosomes are undergoing abnormal division (carc. rectum). × 1,800.
FIG. 12.—Three adjacent dividing cells in metaphase with 24 chromosomes (carc. rectum). × 2,000.
FIG. 13.—Two dividing daughter cells with 32 chromosomes. The chromosomes of the cell on the right are undergoing fragmentation (carc. rectum). × 2,000.

TABLE I.—*The Frequency of Mitotic Abnormalities in Cell Populations taken from the Periphery and Centre of the Same Tumour Cord.*

Tumour.	Periphery. Region (multiple biopsies).	Periphery. Number of dividing cells.	Periphery. Percentage of abnormal dividing cells.	Centre. Region (multiple biopsies).	Centre. Number of dividing cells.	Centre. Percentage of abnormal dividing cells.
Squamous-cell carcinoma cervix No. 41	A	151	3·97	E	51	15·67
	B	97	5·15	F	48	20·83
	C	241	5·80	G	93	24·74
	D	119	5·88	H	131	29·77
Total	..	608	5·26	..	323	24·76



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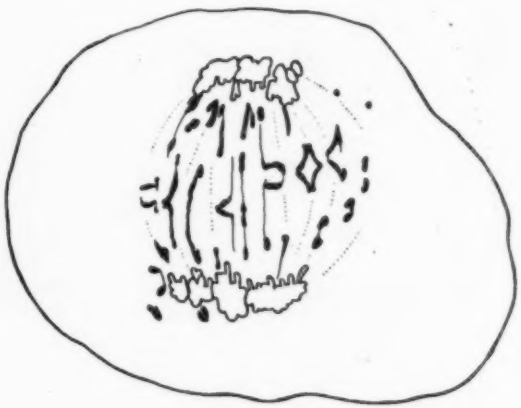

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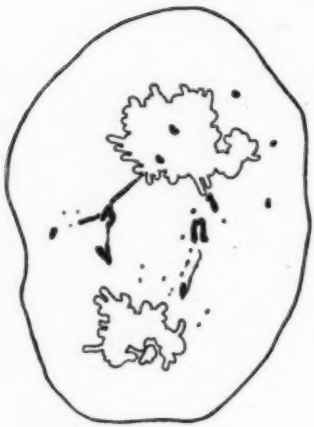
FIG. 14.—Telophase of mitosis showing stickiness of chromosomes. The cytoplasm stains with methyl green pyronin, particularly in the centre of the cell, where the equatorial plate is located during metaphase (carc. cervix). × 1,800.

FIG. 15.—Sticky chromosomes at telophase simulating the bivalent configuration of meiosis (carc. cervix). × 1,800.

The mitotic abnormalities which occur naturally in tumours can be divided into three classes: (i) Structural alterations in the chromosomes; (ii) numerical changes in the chromosome complement; and (iii) complete or partial suppression of the spindle.

(i) Amongst the changes which affect chromosome structure, most common is stickiness of the chromosomes, which can be easily observed during anaphase (Fig. 14, 15).* The chromosomes stick together at the end and lag behind the normally segregating chromosomes. Sticky chromosomes form bridges stretching between the poles, thus preventing the separation of the telophase chromosome groups (Fig. 2, 3, 4). Cell division very often remains incomplete (Fig. 17, 18, 19, 20, 21). Loss of chromosome material in such cells occurs very frequently.

* Fig. 14 to 27 are camera lucida drawings of cells from squash preparations. Technique is described by Koller (1942).



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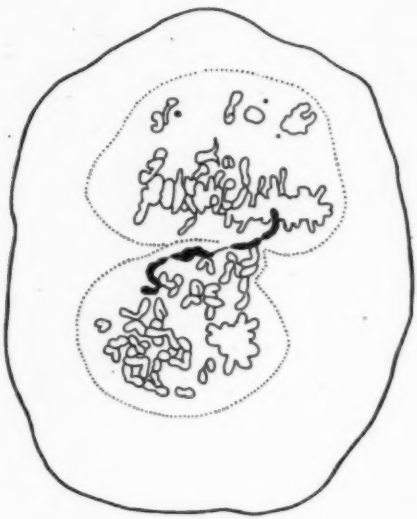

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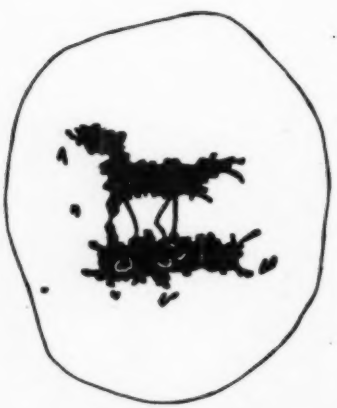
FIG. 16.—Tumour cell in late telophase, showing lagging of sticky chromosomes and small granules of chromosome material which were shed during anaphase (carc. skin). × 1,800.

FIG. 17.—Mitosis in a binucleate cell in which the two nuclei are incompletely separated (carc. breast). × 1,800.



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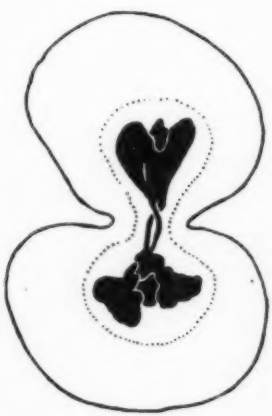

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FIG. 18.—Multiple chromosome bridges and displaced chromosomes at late anaphase (carc. cervix). × 1,800.

FIG. 19.—Incomplete cell division due to stretched out chromosome bridges which prevent the separation of the daughter nuclei (carc. cervix). × 1,800.

The size of chromosome fragments shows great variation (Fig. 16). Often chromosomes may be caught between the bridges, in which case they will be lost (Fig. 20). The abnormal chromosome configuration, which results from stickiness, very closely resembles the bivalent of meiosis (Fig. 14, 15). This similarity led Farmer, Moore and Walker (1906) erroneously to assume that carcinogenesis is a process similar to gametogenesis, which takes place abnormally in somatic tissues.

Quantitative analysis of tumours of various kinds shows that the number of cells with sticky chromosomes differs significantly (*a*) in different tumours, and

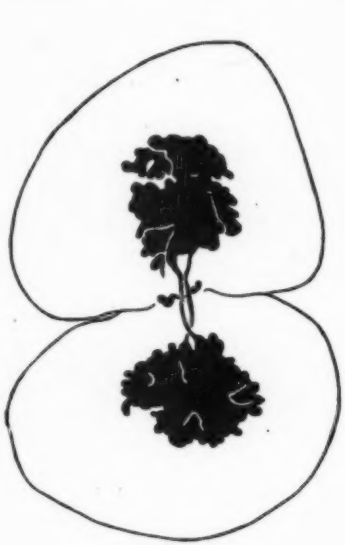

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FIG. 20.—Incomplete telophase, showing elimination of a chromosome caught between two chromosome bridges (carc. skin). × 1,800.

FIG. 21.—Late telophase showing a very thin chromosome bridge (adenocarc. uteri). × 1,800.

(*b*) in different regions of the same tumour (Table II). Comparison of data obtained from biopsies taken from the same region of a tumour at different intervals suggests that the frequency of abnormally dividing cells in the same cell population can also fluctuate. On account of such variability, which cannot be predicted, it is not permissible to use the frequency of cells with abnormal mitosis as a criterion for estimating either the growth-rate or the degree of malignancy of tumours.

It has been shown that nucleic acids play an important part in the organization of chromosomes during cell division. Nucleic acid makes the chromosomes visible and enables them to contract during the prophase of mitosis. Stickiness and over-condensation (Fig. 6) is attributed to an excess of nucleic acid charge on the chromosomes (Darlington, 1942).

TABLE II.—*Frequency of Mitotic Abnormalities in Different Tumours and in Different Regions of the Same Tumour.*

Skin.			Cervix and corpus uteri. (Single biopsies.)			Cervix No. 1. Squamous carcinoma. (Multiple biopsies.)		
Tumour.	Cells in mitosis.		Tumour.	Cells in mitosis.		Region.	Cells in mitosis.	
	Total number.	Percentage abnormal.		Total number.	Percentage abnormal.		Total number.	Percentage abnormal.
Squam. cell carc. (cheek)	135	2·23	Squam. cell carc.	132	3·03	A	51	5·89
Squam. cell carc. (forehead)	121	6·62	Squam. cell carc.	145	6·89	B	75	6·66
Basal cell carc. (forehead)	141	9·22	Adenocarc.	150	14·00	C	71	8·46
Kerat. squam. cell carc. (outer canthus)	102	11·77	Squam. cell carc.	570	16·11	D	63	9·53
Squam. cell carc. (hand)	153	16·99	Ditto.	150	16·67	E	97	14·44
Basal cell carc. (cheek)	137	17·52	„	165	23·63	F	92	15·22
						G*	121	38·85
$\chi^2_{(5)} = 31{\cdot}45127$ $P = >0{\cdot}001$			$\chi^2_{(5)} = 68{\cdot}8522$ $P = >0{\cdot}001$			$\chi^2_{(6)} = 58{\cdot}7677$ $P = >0{\cdot}001$		

* This region showed extensive necrosis.

(ii) Cells with an abnormal chromosome number were observed in tumours of mice (Goldschmidt and Fischer, 1929; Winge, 1930) and man (Kemp, 1930; Koller, 1943*b*). If the chromosome number is doubled, the cells are larger than those with the normal diploid chromosome number. Polyploidy, or increase in the chromosome number, usually arises through the failure of spindle formation (Fig. 5). Complete suppression of spindle is common in cells in which chromosome synthesis is extremely rapid. Comparison of the frequency of cells in various stages of mitosis in normal and tumour tissues has shown that chromosome synthesis proceeds at a higher rate in tumour cells than in normal cells (Koller, 1947). On that account, spindle abnormalities in tumour cells can be expected.

Stickiness and lagging of chromosomes during anaphase occasionally results in nuclei in which some chromosomes are absent, or in which extra chromosomes are included in addition to the normal forty-eight (Fig. 22). It seems that cells with an unbalanced chromosome number are more common than the balanced polyploid cells. Recently evidence was obtained which suggests that polyploidy may arise through the process of endomitosis, i.e. by repeated chromosome multiplication within the nuclear membrane (Biesele, Poyner and Painter, 1942; Koller, 1943*c*).

Polyploid cells are frequent in tumour regions where cell breakdown is in progress on a large scale. From the necrotic slough of carcinoma of the cervix and from the aspirated fluid of carcinoma of the alimentary canal, giant cells can be separated by centrifugation. They contain a very high number of chromosomes, which are usually small and lie scattered throughout the cytoplasm (Fig. 9). The number of chromosomes may be so high that the degree of polyploidy of the cell cannot be determined (Koller, 1943*b*). Biesele *et al.* (1942) reported a cell with 885 chromosomes which was seen in methylcholanthrene-induced

mouse sarcoma. The normal diploid number being 40, some of the chromosomes must have been present in this particular cell more than 32 times. The high frequency of cells in tumours with an irregular number of chromosomes led Boveri (1914)—again erroneously—to attribute malignant cell behaviour to an unbalanced chromosome number.

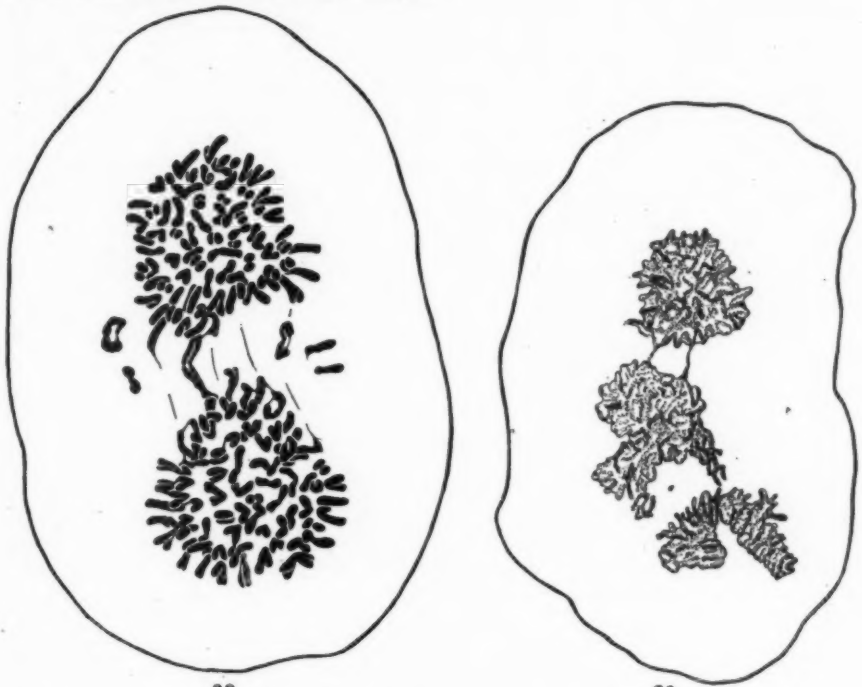

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FIG. 22.—Metaphase in binucleate cell. The chromosomes of the two daughter nuclei form separate equatorial plates (carc. skin). × 1,800.
FIG. 23.—Multinucleate cell at the end of telophase. The daughter nuclei remain in association by sticky chromosome bridges (carc. cervix). × 1,800.

(iii) The centromere of chromosomes and the centrosomes are primarily responsible for the organization of the spindle (Darlington, 1940), but the cytoplasmic environment also plays an important rôle in its formation (Bernal, 1940; Östergren, 1944*a*). Extra division of centromeres and abnormal orientation of metaphase chromosomes often leads to the formation of multipolar or incomplete spindle (Fig. 7, 24 to 27). The former is specially common in polyploid cells; the latter develops in multinucleate cells undergoing synchronous division (Fig. 8). The unipolar spindle is rare (Fig. 27). Stickiness of anaphase chromosomes is usually accompanied by the presence of an incomplete or abnormally oriented spindle (Fig. 4 and 25). The daughter nuclei, after the completion of multipolar mitosis, often remain associated by chromosome bridges (Fig. 17 and 23).

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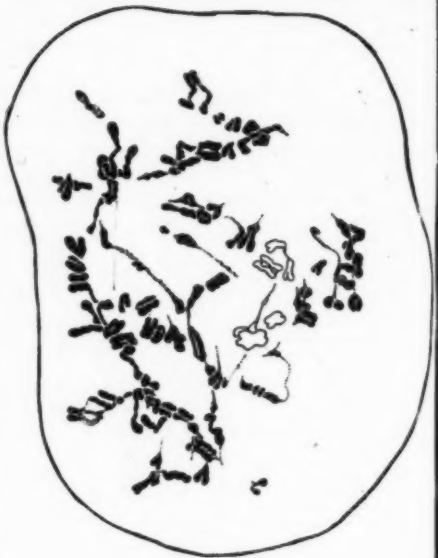

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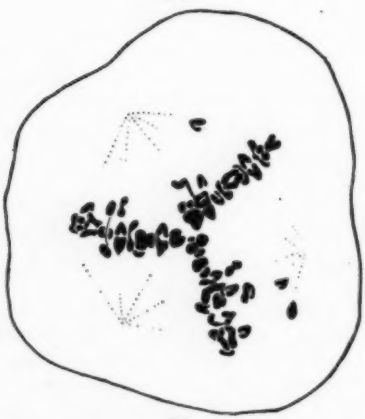

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FIG. 24 and 25.—Polyploid tumour cells with incomplete multipolar spindles and sticky chromosomes (carc. cervix). × 1,800.

FIG. 26.—Tri-polar spindle at metaphase (carc. breast). × 1,800.

FIG. 27.—Uni-polar spindle with sticky chromosomes (carc. skin). × 1,800.

The abnormalities of all three categories described above can be brought about experimentally in normal cells by various external agencies. X-rays induce stickiness of chromosomes in plants and animals (Markquand, 1938; Koller, 1943*a*; Darlington and La Cour, 1945). Temperature changes and chemical treatment can lead to similar abnormalities (Barber and Callan, 1943; Östergren, 1944*b*), and complete suppression of the spindle is brought about by low temperature and by specific compounds, such as colchicine.

Polyploid, binucleate and multinucleate cells, owing to their chromosome balance, survive and may undergo mitosis. Their rate of mitosis, however, is always less than that of cells with a normal chromosome number. On account of their lower frequency and low rate of division, they play an insignificant role in the growth of the tumour. Polyploid cells are, however, more resistant to irradiation by X-rays than diploid cells, and under specific histological conditions they can be responsible for tumour recurrence (Koller and Smithers, 1946).

INTERNALLY CONDITIONED ABNORMALITIES.

Nuclei with complete chromosome sets are necessary for the regular functioning of cells (Koller, 1943*a*), and experimental proof is available to show that loss of chromosomes or of chromosome segments leads to the death of the cell (Koller and Smithers, 1946). Cells with deficient nuclei can survive only under very specific conditions (Barber, 1941; Sax, 1942), although such cells have been observed in leukaemic bone marrow of mice and in the normal bone marrow of the fowl (Koller, unpublished).

The frequency of chromosomally unbalanced cells is, however, low and their distribution in tissues sporadic. In the course of cytological investigations of human tumours, several were found with regions in which cells with an irregular chromosome number were very abundant. Cell and chromosome behaviour was analysed in detail in one such tumour, an adenocarcinoma of the rectum (Fig. 10 to 13).

The chromosome number showed great variation (Fig. 28). Cells with 36, 32, 24 and 16 chromosomes were the most numerous. It was observed that the chromosomes were longer and more slender than in normal diploid cells. In some cells the chromosomes appeared as thin, lightly-stained threads. As a general rule there was synchronization in behaviour of adjacent cells. In one region, 16 cells were seen in simultaneous mitosis. Chromosomes, and occasionally whole chromosome sets, were found to fragment. The breakage occurred in chromosome regions which were thin and understained with Feulgen's basic fuchsin (Fig. 13). It was found that the rate of mitosis of the chromosomally unbalanced cells was higher than the rate of breakdown of such cells.

The peculiar chromosome behaviour in this adenocarcinoma throws new light on the relationship of tumour cells. Sufficient evidence is available from experimental tumour transplantation, tissue culture work and tumour pathology to show that adjacent tumours cells may still be dependent on each other in many respects, and the adenocarcinoma in question illustrates to what a great extent this may be so. There is no direct evidence as to the mechanism responsible for synchronous division, but it seems conceivable that a specific substance, necessary for chromosome synthesis, is transferred from one cell to adjacent cells (Barber, 1941; Sax, 1942).

Until recently it was believed that a nucleus with the diploid chromosome number was necessary for normal cell behaviour, and that cells with a deficient complement could function only under very specific conditions, the life of such cells always being short (Darlington, 1942). The adenocarcinoma here described presents an example in which cells with less than the normal number of chromosomes are still able to divide and to continue dividing. It is not out of place to draw attention to recent literature on cytoplasmic control in micro-organisms (Sonneborn, 1943 ; Spiegelman and Kamen, 1946), the relevance of which, for the origin of cancer, has been dealt with by many authors (Graffi, 1939, 1940*a*, *b* ; Darlington, 1944 ; Haddow, 1944 ; Potter, 1945 ; Woods and Du Buy, 1945, 1946), who suggest that the permanent change which renders a cell malignant takes place in the cytoplasm. Cell behaviour in this adenocarcinoma is a further indication that the nucleus can be so subordinated, and the cell remain active in spite of its deficient nucleus.

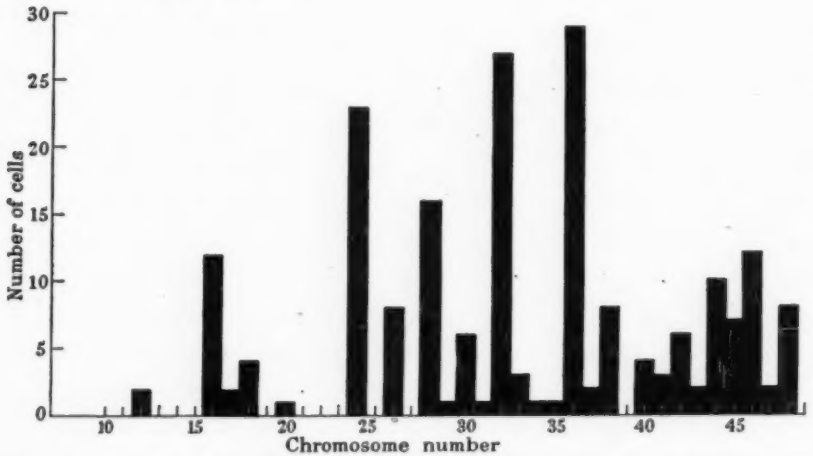

FIG. 28.—Diagram showing the distribution of cells with different chromosome numbers (200 mitoses).

SUMMARY.

1. The most common mitotic abnormalities naturally occurring in tumours consist of stickiness of chromosomes, irregular or polyploid chromosome number, and incomplete or complete suppression of the spindle.

2. These abnormalities are partly attributed to lack of food supply and to toxic breakdown products, both of which are always present in rapidly growing tumour regions.

3. Beside the abnormalities due to environmental factors, a large number of active tumour cells with a reduced chromosome number may also be seen in some tumours. In undergoing mitosis such cells may exhibit great dependence on each other.

4. The behaviour of tumour cells with a reduced chromosome number suggests that mitotic activity is under cytoplasmic and not nuclear control.

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THE CYTOLOGICAL ACTION OF ETHYLCARBAMATE (URETHANE) AND OTHER CARBAMIC ESTERS IN NORMAL AND LEUKAEMIC MICE, AND IN RABBITS.

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FOLLOWING the discovery by Haddow and Sexton (1946) of the effects of ethylcarbamate (urethane) on the growth and differentiation of the Walker rat carcinoma, the action of this substance in human leukaemia was described by Paterson, ApThomas, Haddow and Watkinson (1946). Since the early experiments of Warburg in 1910, ethylphenylcarbamate (phenylurethane) had been known to arrest the division of the sea-urchin egg at metaphase. A similar effect in plant cells, closely resembling that of colchicine, was discovered in 1939 by Lefèvre and by Simonet and Guinochet (1939), and was further studied by Gavaudan (1943) and Deysson (1944). Ludford (1936) treated tissue cultures with a 2 per cent solution of ethylcarbamate and noted abnormal metaphases and a considerable amount of nuclear and cellular degeneration. Similar results were obtained by Geiersbach (1939), and Östergren (1944) also claimed that ethylcarbamate arrested mitosis at metaphase in *Allium*. Templeman and Sexton (1945), investigating the growth-inhibiting properties of numerous carbamic esters in plants, found, however, that ethylcarbamate was inactive in this respect, although marked activity was shown by ethyl phenylcarbamate and isopropyl phenylcarbamate. It was this work which led Haddow and Sexton to investigate the action of carbamic esters on tumour growth, and to the discovery that in this case ethylcarbamate itself is effective. These authors suggested that this substance might belong to the group of caryoclastic (nuclear) poisons studied by A. P. Dustin. The purpose of the present paper was to follow this hypothesis. In consequence, the organs in which these caryoclastic effects are known to be most easily observed in mammals (thymus and lymphoid tissue, intestinal mucosa) were specially studied. Experiments were also directed towards investigating the resultant blood changes in normal and leukaemic mice.

MATERIAL AND METHODS.

Experiments were carried out using stock mice (20–25 g.) and mice of the C57 pure line (*c.* 15 g.). In the latter, an acute leukaemia H.R. described by Gorer (1946) was utilized, in which large stem-cells produced a diffuse infiltration of lymph nodes, spleen, liver and kidneys, leading to death of the mice in from 14 to 22 days after subcutaneous grafting of leukaemic tissue. Enumeration of reticulocytes was made from dry blood smears, following postvital staining with

* Associé du Fonds National Belge de la Recherche Scientifique. Now Lady Tata Memorial Fund Fellow.

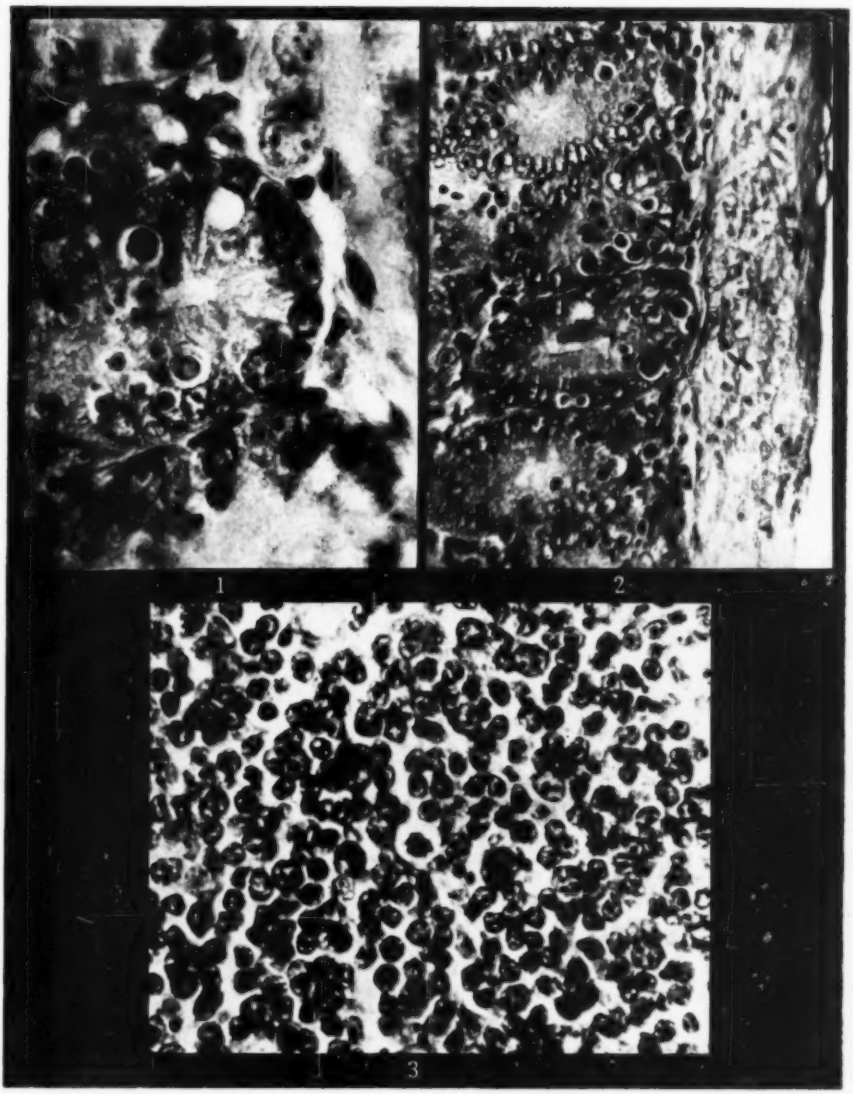

FIG. 1.—Intestinal crypts of mouse 10 hours after intraperitoneal injection of 20 mg. (1 g./kg.) of ethylcarbamate. No normal mitoses; extensive nuclear destruction.

FIG. 2.—Intestinal crypts of mouse 24 hours after intraperitoneal injection of 20 mg. (1 g./kg.) of ethylcarbamate. Nuclear destruction and recovering mitosis.

FIG. 3.—Cortical region of the mouse thymus 8 hours after intraperitoneal injection of 50 mg. (2·5 g./kg.) of ethylcarbamate. Normal mitosis; no pyenoses.

brilliant cresyl blue (Dustin, Jr., 1944). Bouin's fluid was used as a routine histological fixative, and cell divisions in the intestine were always studied in the first jejunal loop. For the examination of mitosis in bone marrow, La Cour's (1944) lacmoid-acetic acid staining of squash preparations was found most useful. This method was also applied to other tissues, in particular the intestinal mucosa.

RESULTS.

1. *Ethylcarbamate.*

(a) *Action of a single injection of ethylcarbamate on mitosis in the tissues of adult mice.*

The effects of a single dose of 1 g./kg. of body-weight will first be described. This dose has a narcotic effect lasting more than 3 hours. Freshly-prepared solutions of ethylcarbamate in distilled water were injected intraperitoneally. The most marked changes take place in the germinative zones of the Lieberkühn glands of the small intestine, and these were studied 2, 4, 6, 8, 10, 24 and 48 hours after injection. Three variables must here be considered, namely the mitotic index, the relative percentage of different mitotic phases, and the number of nuclei undergoing degeneration.

(i) One of the best methods for estimating the approximate number of dividing cells in the intestinal mucosa is to count 100 consecutive glandular crypts. This is more reliable than counting the mitoses in a certain number of microscopic fields. In normal mice, in sections 5 to 7μ thick, 1·2 to 1·5 divisions are found per crypt. After the injection of urethane, this figure does not change during the first 8 hours, but falls to about 0·5 at 10 hours. This decrease in mitotic activity persists 24 hours after the injection. Normal figures are found after 48 hours. Thus ethylcarbamate temporarily lowers the total number of cells undergoing mitosis in the intestine.

(ii) During these experiments there is no evident change in the percentage of different mitotic phases. In one series of mice, prophases varied between 13 and 15 per cent; metaphases, 32 and 52 per cent; ana- and telophases together, 42 and 56 per cent. This indicates that ethylcarbamate does not poison any given phase of cellular division; there is no metaphase arrest of the "colchicine-type." The mitoses which take place are morphologically normal, apart from the possible inclusion of cellular fragments.

(iii) In control animals, occasional pycnotic and shrunken nuclei may be found in the germinative zone of the intestine. Their number increases considerably after a single injection of ethylcarbamate (1 g./kg.):

2 *and* 4 *hours.*—Normal divisions; no degenerating nuclei in abnormal numbers.

6 *hours.*—Slight increase in nuclear destruction.

8 *hours.*—Several pycnotic nuclei in each crypt.

10 *hours.*—Numerous (up to 10 and more) pycnoses per gland (Fig. 1).

24 *hours.*—The nuclear fragments are very numerous. They fuse together and fall into the glandular lumen, being thus eliminated from the epithelium. Fragments are also engulfed in the cytoplasm of normal cells (Fig. 2).

48 *hours.*—The epithelial divisions are again normal, nearly all pycnoses have disappeared, and the only sign of the ethylcarbamate effect is the occasional presence of some voluminous nuclear fragment not yet pushed out into the intestinal cavity.

Nuclear destruction takes place with marked decrease in mitotic index, suggesting that the nuclear fragments originate from cells which normally would have divided. This is substantiated by the cytological analysis of this phenomenon. In normal division, the cells of the intestine undergo the following changes:—*Prophase :* The resting nucleus, lying against the basal membrane, swells progressively and the chromosomes appear close to the nuclear membrane. *Metaphase :* The breakdown of the nuclear membrane is accompanied by marked swelling of the whole cytoplasm, and, as a consequence, the cell migrates towards the glandular lumen. *Ana-* and *Telophase :* The spindle is oriented parallel to the glandular axis. The daughter-nuclei are bean-shaped, and markedly smaller than the resting nuclei* ; they progressively regain the basal zone.

When the nuclei undergo degeneration, the following phases may be observed (Fig. 1): In a cell with a resting nucleus, the cytoplasm undergoes marked swelling. This takes place in a very short time (probably a few minutes), and often brings the cell, as in metaphase, towards the centre of the gland. The nucleus then disintegrates, the thymonucleic acid material forming shell-like structures covering an acidophil sphere, which also contains some ribonucleic acid (by the ribonuclease method) and the remnant of the nucleolus. Further destruction leads to irregular fragments, with a positive or negative Feulgen reaction. These are easily engulfed in the cytoplasm of neighbouring cells, and may sometimes be found in dividing cells.†

Whatever the mitotic poison, these nuclear effects are only to be found in the region where mitosis normally takes place, and the more superficially located resting nuclei are not damaged. This provides evidence that the destruction only affects those cells which would normally have undergone division.

In other tissues no similar changes are to be found. The lymphocytes in the thymus are known to be the most sensitive cells towards mitotic poisons, yet even after injection of 2·5 g./kg. of ethylcarbamate their structure and division appear normal (Fig. 3). In the spleen, neither the divisions of the lymphocytes nor those of the erythroblasts and myelocytes are visibly affected. In the bone-marrow, a study of the mitotic phases was made at repeated intervals after the injection of 1 g./kg. of ethylcarbamate. After 10 hours there were 13 per cent prophases, 63 per cent metaphases and 24 per cent ana- and telophases. After 24 hours the figures were respectively 15, 66 and 19 per cent. The total number of divisions was not estimated and may have varied, as further experiments will show.

The intestinal mitoses appearing to be the most sensitive, an attempt was made to determine the threshold dose of ethylcarbamate. The mitoses and pycnoses were studied 24 hours after the injections, and the results in Table I show that nuclear destruction, already apparently increased by 0·1 g./kg., is considerable after 0·2 g./kg.

* This is perhaps one of the facts which misled Andrew and Andrew (1945) to consider these mitoses as belonging to lymphocytes migrated within epithelial cells.

† This has also been described by Spear and Glücksmann (1938) in the retina of irradiated tadpoles.

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No other significant changes were observed in other organs after a single injection of ethylcarbamate.

TABLE I.—*Threshold Action of a Single Intraperitoneal Injection of Ethylcarbamate on the Intestinal Mitoses of the Mouse.*

Each figure represents the total of 100 consecutive glands, at 24 hours following injection.

	Dose of ethylcarbamate (g./kg.).			
	0·20	0·15	0·10	0·05
Mitoses	147	147	210	181
Pycnotic fragments	154	39	23	8

(b) *Action of a single injection of ethylcarbamate on the number of reticulocytes in rabbit's blood.*

The most sensitive index of the mitotic activity in the red blood cell forming tissues is the reticulocyte percentage in the circulating blood, as pointed out by Langendorff (1938) and Mardersteig (1938) in the study of X-rays on haemopoiesis. The rabbit was chosen as experimental animal for technical reasons, and because marked changes in the reticulocyte percentage had previously been found after the injection of colchicine (Dustin, Jr., 1941).

A single (subcutaneous or intraperitoneal) injection of 0·25 g./kg. of ethylcarbamate did not have any effect, but doses of 0·8 and 1·0 g./kg. produced a definite fall in the number of reticulocytes (Fig. 4 and Table II). In six experiments (five rabbits) this fall was found 48 hours after injection. It was transient,

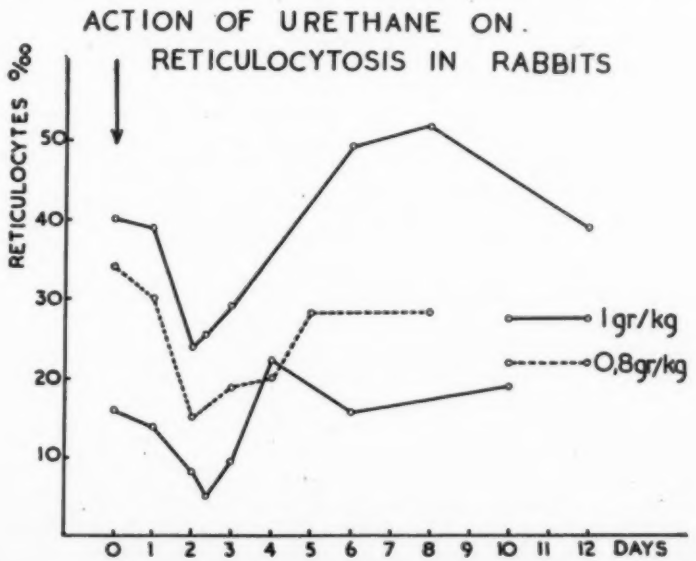


FIG. 4.

TABLE II.—*Action of Ethyl- and of Methylcarbamate on the Reticulocytes (‰ Red Blood Cells) of Rabbit's Blood.*

Rabbit No.	Injected (g./kg.).	Days after injection. 0.	1.	2.	3.	4.	5.	6.	7.	8.
	Ethylcarbamate.									
3	0·5	31	57	30	39	..	..	..	..	..
2	0·8	34	30	*15*	19	20	28	..	..	28
1	1·0	16	14	*8*	9	22	..	16	..	12
2	1·0	40	39	*24*	28	..	..	49	..	52
5	1·0	24	14	*16*	*11*	22	..	..	20	..
6	1·0	30	37	*12*	*11*	20	..	..	21	..
7	1·0	22	16	*13*	18	15	..	..	..	..
	Methylcarbamate.									
2	2·0	17	26	22	24	..	..	..	..	..
3	2·0	15	..	..	..	19	..	..	18	..

and within 3 days the number of reticulocytes was close to the initial value. No significant change in the numbers of red blood cells, or in the leucocytes, was observed.

These results may be compared with those following the injection of colchicine, when the reticulocyte fall is marked and the minimum is also reached in 48 hours. The erythroblastic mitoses are arrested in metaphase by colchicine during the first hours following its injection, and the delayed decrease of the number of reticulocytes is a consequence of the time needed for the maturation of the erythroblasts. The similarity between the curves for colchicine and for ethylcarbamate suggests that during the first day the narcotic has slowed the production of red blood cells. This is in agreement with the findings for the intestine of the mouse. The fact that neither in rabbits nor in mice can this action of ethylcarbamate be detected by histological methods is only a consequence of the difficulty of estimating the total number of mitoses taking place. The possibility of a mitotic arrest at metaphase is ruled out, but any arrest before prophase would be very difficult to demonstrate by histological methods, especially if there is no extensive cellular breakdown as in the intestine.

(c) *Action of repeated injections in normal and leukaemic C57 mice.*

Apart from their specific effects (for instance mitotic poisoning in the intestine), daily injections of ethylcarbamate may affect the general nutrition of the animals, and cause loss of weight. Underfeeding is known to lead to atrophy of the lymphoid organs (in particular the thymus), and also to slow malignant growth. Hence the great importance of dissociating the direct effects of carbamic esters from non-specific changes related to loss of weight.

(i) *Effect on body-weight.*—Daily injections of 0·25 g./kg. given for a period of two weeks were not found markedly to affect the weight of normal or leukaemic C57 mice. The average loss was about 1 g. for two groups of 5 mice (normal, 17 to 16·3 g.; leukaemic, 19 to 17·9 g.). The same experiment with 0·5 g./kg. daily showed conflicting responses. Some mice lost several grams in a few days,

usually dying after losing about one-third of their weight. Others remained in good condition, but lost on the average 2 grams. In a few mice no change of weight was observed. By comparing the histological pictures found in these last two groups it is possible to appreciate the specific effects of ethylcarbamate.

(ii) *Blood-picture.*—The principal changes were an increase in the percentage of granulocytes and a decrease in the numbers of reticulocytes. As could be expected from the results obtained in the rabbit, only doses of 0·5 g./kg. daily changed the reticulocytosis.

Tables III and IV show the influence of repeated injections and ingestion of ethylcarbamate on the reticulocytes of normal and leukaemic C57 mice.

TABLE III.—*Daily Injection of 0·5 g./kg. of Ethylcarbamate. Reticulocytes ‰ Red Blood Cells (500 Cells Counted).*

Mouse No.	Before 1st injection.	6 days.	11 days.	14 days.	Total loss of weight after 14 days (g.).
1	28	22	56	96	2
2	48	11	6	22	4
3	22	6	28	72	1
4	64	14	9	38	4
5	42	16	15	22	1

TABLE IV.—*Reticulocytes (‰ Red Blood Cells) after 10 Daily Injections of 5 mg. Ethylcarbamate (c. 0·7 g./kg.) or Feeding with Ethylcarbamate.*

	Controls normal.	Non-leukaemic fed ethyl-carbamate.	Leukaemic fed ethyl-carbamate.	Leukaemic injected.	Leukaemic controls.
	28	23	36	7	114
	31	82	13	6	82
	54	18	14	22	84
	72	10	17	6	62
	34	16	7	10	100
Average	44	30	17½	10	88½

This was most conspicuous in leukaemic animals, where the reticulocytosis associated with the neoplasia was no longer visible (Table IV). Table III shows that the reticulocyte fall may be transient, and followed by a rise even when the daily administration of ethylcarbamate is pursued. It did not appear to be related to loss of body weight, as is the following effect. With daily doses exceeding 0·5 g./kg., extremely low reticulocyte numbers (sometimes less than 1‰) were found, both in normal and leukaemic mice. This was not an indication of irreversible damage to red blood cell production, and after cessation of the injections a high reticulocytosis was found. The blood of normal C57 mice contains about 30 per cent of granulocytes, and a similar proportion is found during the leucocytosis associated with the H.R. leukaemia. In 1925 Hawkins and Murphy found that urethane anaesthesia was followed by a relative increase

in the percentage of granulocytes in rabbits. Both in normal and leukaemic mice the continued administration of ethylcarbamate has the same result. Though individual differences may be great, in all animals treated with at least 0·5 g./kg./day, the percentage of granulocytes was increased after 15 injections to an average figure of 60 per cent both in normal and leukaemic mice. The change is quite conspicuous after 6 daily injections. No apparent relation was found between the percentage of granulocytes and the total number of leucocytes, which, particularly in leukaemic animals, varied considerably (between normal figures averaging 5000, and 120,000/c.mm.).

(iii) *Organ weights and histology.*—Table V shows clearly that both in normal and leukaemic animals ethylcarbamate produces a marked atrophy of the spleen, which is associated with thymic atrophy. There is some evidence that the growth of the H.R. leukaemic cells is also disturbed, as evidenced by the smaller size of the tumour at the site of grafting and the less conspicuous enlargement of liver, spleen and kidneys by neoplastic infiltration. This effect seems partly non-specific, and can be produced by underfeeding alone.

The following histological changes are to be found in mice given repeated injections of ethylcarbamate (0·5 g./kg.) for 10 to 15 days without loss of weight :

Thymus : the cortical zone is thinner than normal, and its limits are less precise. Normal mitoses are found in the cortex, and there is no evidence of increased pycnotic destruction of thymocytes.

Spleen : the atrophy of the organ affects all haemopoietic tissues. Germ-centres are rarely present in the lymph follicles. Formation of granulocytes, megakaryocytes and red blood cells is still taking place.

Lymph-nodes : slight atrophy.

Bone-marrow (femur) : very active granulopoiesis. No evidence of aplasia or atrophy.

Liver, Kidneys, Adrenals : no histological change.*

Intestine : much pycnotic debris in all Lieberkühn crypts, side by side with normal mitoses. No evident atrophy of the mucosa, nor ulceration.

Leukaemic infiltrations : no evidence of increased cell-destruction nor mitotic abnormalities.

In animals losing weight during the experiment, splenic and lymphoid atrophy is more pronounced. The formation of granulocytes and red blood cells in the spleen is markedly reduced. The bone-marrow remains very cellular, with a high proportion of immature granulocytes. There is no evidence of aplasia. In the thymus the atrophy of the cortical region is complete, the epithelial cells only remaining. This picture of " inverted thymus " is found after the pycnotic destruction of thymus cells by mitotic poisons (Dustin, 1929) or non-specific stimuli (Selye, 1937), and in inanition. In this case it is probable that the ethylcarbamate injections and the loss of body weight have additive effects.

No marked changes are found in other organs.

* Rosin and Doljanski (1944) have described inclusions in liver cells of rats treated with urethane, which they believe are red blood cells engulfed in cytoplasm and even in the nucleus. From the evidence given, the origin of these inclusions is doubtful. Similar bodies were described in the liver cells of the mouse by Campbell (1939) and observed occasionally in our material.

TABLE V.—*Action of Ethylcarbamate and of Underfeeding on the Organ and Body Weights of C57 Normal and H.R. Leukaemic Mice.*

Group.	Weight (g.). Initial.	Weight (g.). Final.	Liver (mg.)	Kidneys (mg.)	Spleen (mg.)	Local tumour + local lymph node metastasis (mg.)
I	..	15·8	925	200	107	..
II	18	16·4	1088	234	*52*	..
III	14·6	16·6	1230	228	104*	540
IV	15·6	13·6	768	188	*42*	*224*
V	14·8	10·2	752	158	*34*	*155*

* The absence of leukaemic enlargement of the spleen contrasts with the results of Table VII. This experiment was the 21st transfer.

The figures are the averages of 5 animals of each group :

I. Normal C57 mice.

II. Normal C57 mice injected 2 × 6 days 0·5 g./kg. ethylcarbamate.

III. H.R. leukaemic mice.

IV. H.R. leukaemic mice injected 2 × 6 days 0·5 g./kg. ethylcarbamate.

V. H.R. leukaemic mice underfed.

Animals of Groups II to V were sacrificed 15 days after the beginning of the experiment.

(d) *Action of prolonged administration of ethylcarbamate in the rabbit.*

The fall in the percentage of reticulocytes after a single injection of 1 g./kg. in the rabbit, and similar findings in mice after repeated injections of more than 0·6 g./kg. of ethylcarbamate, suggested that aplastic changes might be induced in prolonged experiments.

Ethylcarbamate was given by forced feeding or daily intraperitoneal injections. Table VI gives the blood-changes found in a rabbit injected with 0·5 g./kg. daily for 5 days, and then 1 g./kg. during 11 days. The spleen was atrophic (525 mg.), the bone-marrow normal. The principal change was an increase in the relative number of polynuclear leucocytes. The absence of reticulocyte fall is notable, and this apparent contradiction with the results of Table II may be due to the relatively small doses used during the first period of the experiment.

TABLE VI.—*Haematological Changes in Rabbit given 5 × 0·5 g./kg. and 11 × 1 g./kg. of Urethane by Intraperitoneal Injection.*

Date.	Red blood cells (millions).	Reticulocytes (‰).	Leucocytes (per c.mm.).	Leucocyte percentages. Neutrophils.	Eosinophils.	Basophils.	Lymphocytes.	Monocytes.
Aug. 12	6·09	11	10,400	15	6	1	83	1
,, 13	6·19	16	8,600	20	..	1	78	1
,, 16	..	18	9,400	24	1	5	70	..
,, 19	6·30	10	12,800	37	1	5	56	1
,, 21	..	22	10,400	34	3	3	60	..
,, 23	6·39	19	9,400	47	..	2	47	3
,, 27	6·75	56	12,400	51	..	4	44	1
,, 29	7·05	36	4,200	59	..	4	32	5

In a rabbit fed 0·5 g./kg. for 5 days, there was an increase in leucocytes from 9200 to 22,800, and in granulocytes from 33 to 58 per cent. The bone-marrow showed very active granulopoiesis.

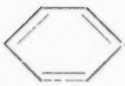
These preliminary results indicate that large doses of ethylcarbamate do not bring about any aplasia of the bone-marrow in the rabbit.

2. *Other Carbamic Esters.*

Some experiments were done with the following carbamic esters:

$NH_2.CO.O.CH_3$
methylcarbamate.

$NH_2.CO.O.CH_2CH_2.Cl$.
β-chloroethylcarbamate.



$—NH.CO.O.C_2H_5$

ethylphenylcarbamate
(phenylurethane).

$—NH.CO.O.CH.(CH_3)_2$

isopropylphenylcarbamate.

Methylcarbamate is readily soluble in water and of low toxicity. In the mouse no histological changes and, in particular, no mitotic disturbances or pycnoses in the intestinal cells, were found 8 and 24 hours after the injection of 0·75, 1·25 and 2·5 g./kg. In the rabbit, no significant change in the reticulocyte count was observed after the subcutaneous injection of 2 g./kg. (Table II). In neither case was there any narcosis.

β-*Chloroethylcarbamate* is less water-soluble than urethane. In the mouse it was found to affect the intestinal mitoses in the same way (0·5 g./kg.), although the mitotic poisoning is not increased by the halogen atom. No significant changes were found in thymus and spleen.

Ethylphenyl- and isopropylphenylcarbamates were dissolved in arachis or olive oil and injected intraperitoneally in stock mice. They were also given with the food to C57 leukaemic mice. Their toxicity is greater than that of ethylcarbamate, and non-specific nuclear destruction in lymphoid tissue is often found. Phenylurethane (0·5 g./kg.) modifies the division in the intestine in the same way as ethylcarbamate. A decrease in the numbers of intestinal mitoses was found 8 hours after the injection of 0·5 g./kg. of isopropylphenylcarbamate. It was not followed by any marked nuclear destruction.

Table VII shows that isopropylphenylcarbamate may be ingested in large amounts without any toxic signs.

TABLE VII.—*Organ Weights (mg.) at Death of C57 Mice with H.R. Leukaemia.*

Ethylcarbamate and isopropylphenylcarbamate given with food. Each figure represents the average of four animals.

	Controls leukaemic.	Ethylcarbamate (18 mg. approx. daily).	Isopropylphenylcarbamate (28 mg. approx. daily).
Liver	2087	1209	1805
Spleen	548	180	291
Kidneys	426	214	280
Local tumour	673	98	370

DISCUSSION.

The most interesting feature of these experiments is the mitotic poisoning, by the carbamic esters which have been tested, and in particular by ethylcarbamate, in the Lieberkühn glands of the intestine in the mouse. Following a decrease of the total number of mitoses, extensive nuclear and cellular destruction takes place (Fig. 1 and 2). It may be inferred that the poisoned cells break down at the moment they attempt to enter prophase. Indirect evidence—by the fall of the reticulocyte percentage—has also been given that similar disturbances of cell-division may take place in other tissues. This type of mitotic poisoning is shared by many other substances (Dustin, Jr., 1947). Ethylcarbamate has a particularly slow action, and does not affect the cell-divisions in the thymus as markedly as do most of the other poisons of this type (caryoclastic effect). The interference with the pre-prophase period is similar to the effect of strong doses of ionizing radiations ("radiomimetic" action, Dustin, 1929), and may explain the significance of an observation by Hawkins and Murphy (1925) that "rats under urethane anaesthesia, given an exposure to X-ray well below the lethal dose, invariably died between 7 and 14 days later."

Ethylcarbamate was also found, confirming the results of Hawkins and Murphy, to increase the percentage of granulocytes in the blood, without changing the total number of leucocytes.

The possible mechanism of mitotic poisoning has been discussed elsewhere (Dustin, Jr., 1947). Inactivation of enzymes playing a part in the onset of prophase (in particular, in the thymonucleoprotein synthesis) is one possibility, though, as Haddow and Sexton have pointed out, in most biochemical work on the effect of ethylcarbamate on enzymes, concentrations have been used which are far in excess of those which would be found in animal tissues (Johnson, 1938; Quastel, 1939; Keilin and Hartree, 1939; Fisher and Henry, 1944; Ormsbee and Fisher, 1944).

The principal problem is whether the mitotic-poisoning properties of carbamic esters are related to their action on malignant growth, and may explain the results obtained in human chronic leukaemias with ethylcarbamate. It is probably of significance that many mitotic poisons have been demonstrated to slow malignant growth. Some affect mitosis in a way similar to ethylcarbamate, e.g. trypaflavine (Brodersen, 1943) and the nitrogen mustards (Rhoads, 1946). Others arrest and destroy mitosis at metaphase, e.g. colchicine and arsenic (Forkner, 1938). Indeed there is a striking similarity between the effect of arsenic and of urethane in leukaemia in man. Yet in our experiments we have only found marked alterations of cell division in intestinal cells, and only indirect evidence of effects on erythroblastic divisions in animals, with doses far greater than those used in human therapy. The results obtained in man may be related to the properties of malignant growth in chronic leukaemias, where the neoplastic cells are continuously thrown into the blood-stream and continuously destroyed. A slight change in the balance between leucocyte production and destruction may be followed by great changes in the numbers of cells in the circulating blood. Hence the very similar results obtained with radiations, arsenic and ethylcarbamate may be the consequence of the same changes in the rate of the divisions of the malignant leucocytes.

SUMMARY.

1. Ethylcarbamate (urethane) poisons cell division in the intestine of the mouse. A decreased mitotic activity lasting from 8 to 24 hours after a single injection is accompanied by greatly increased cellular degeneration. This effect is similar to that of other mitotic poisons and X-rays. Cells outside the germinative region are not affected, and it is probable that degeneration takes place only when cells attempt to divide.

2. Even with large doses of ethylcarbamate no comparable cellular destruction is found in other organs.

3. A single injection of ethylcarbamate (0·8 and 1 g./kg.) decreases the numbers of reticulocytes in the blood of the rabbit, between 2 and 3 days after injection. The similar changes in reticulocyte count after the injection of colchicine suggest that ethylcarbamate has temporarily inhibited the division of erythroblasts.

4. Repeated injections of ethylcarbamate in mice increase the proportion of granulocytes, and decrease the numbers of reticulocytes. The lymphoid tissues and the thymus are slightly atrophied. Large doses interfere with nutrition, and produce secondary changes related to loss of weight.

5. Ethylcarbamate has some inhibiting effect on the growth of an acute leukaemia in C57 mice.

6. Splenic atrophy and an increase in the percentage of granulocytes are found in the rabbit after repeated injections of ethylcarbamate.

7. The possible relations between mitotic poisoning and the therapeutic action of ethylcarbamate in human leukaemia are discussed.

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INDUCTION OF PROSTATE TUMOURS IN MICE.

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THE object of this investigation was the induction of prostate tumours in mice by 20-methylcholanthrene.

Pure line strains of mice were used so that transplantation of the experimental tumours might lead, if successful, to the continuous propagation of uniform tumours for subsequent experiments on the effects of oestrogen upon tumour growth.

THE STRUCTURE OF THE NORMAL PROSTATE GLAND IN MICE.

The nomenclature of the prostate lobes in rodents has led to much controversy (Rauther, 1903; Disselhorst, 1904; Walker, 1910; Burrows, 1934; Korenchevsky and Dennison, 1935).

The glands, generally referred to as the lobes of the prostate in the mouse, have been described by Deanesly and Parkes (1933), as consisting of paired anterior, ventral and dorsal lobes, together with a small median gland—the ampullary lobe. Fekete (1941) described this tubular median gland as lying adjacent to and opening into the ampulla of the ductus deferens.

It is often suggested that the series of glands comprising the prostate of rodents are not necessarily homologous with the human prostate. Nevertheless, these structures in rodents and in man arise by similar outgrowths from the wall of the urethra, and there are clear histological similarities between the components of the rodent and human glands.

Thus we find in the mouse: (1) The anterior prostates which are paired lobes, sometimes referred to as the coagulating glands (Burrows, 1945), but described by Deanesly and Parkes (1933) as the anterior prostates. They are embedded in the peritoneal sheath of the seminal vesicles, and open into the anterior dorsal wall of the urethra by means of two ducts. They are lined by simple columnar epithelium. The mucous membrane is thrown into folds which project into the

lumen, their appearance depending upon the functional activity of the gland (Fig. 1).

The anterior prostate secretes an enzyme which coagulates the seminal fluid, hence the alternative name " coagulating gland." This gland is considered by some investigators (Callow and Deanesly, 1935 ; Moore, 1939) to be part of the true prostate, because it has a similar embryological origin as the ventral and dorsal prostates. It also possesses the same histological structure as the dorsal lobes.

(2) The dorsal prostates are considerably smaller in size than the anterior glands, having several ducts, all of which open into the dorsal wall of the urethra. Their histological structure is similar to that of the anterior lobes, except that the epithelium of the dorsal lobes is relatively free from folds (Fig. 2).

(3) The ventral prostates are lined by a low columnar epithelium (Fig. 3). There are four ducts, all of which enter the ventral wall of the urethra near the bladder.

METHODS.

These experiments were confined to mice of R III and Strong A strains. The average age of the mice was 6 months. Methylcholanthrene was introduced into the prostate gland in the following manner :

Under general anaesthesia, a median abdominal incision was made, exposing the prostate lobes. Using a dissecting microscope, 0·1 c.c. of a 1·5 per cent solution

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1.—Anterior prostate or coagulating gland of a 6 months' old Strong A mouse. Masson's light green-eosin.

FIG. 2.—Dorsal prostate of a 6 months' old Strong A mouse. Masson's light green-eosin.

FIG. 3.—Ventral prostate of a 6 months' old Strong A mouse. H. and E.

FIG. 4.—Technique by which 20-methylcholanthrene is injected into the dorsal prostatic lobe.

FIG. 5.—Typical tumour of the prostate gland in a Strong A mouse 4 months after the injection of methylcholanthrene.

FIG. 6.—Action of 20-methylcholanthrene on the epithelium of the anterior prostatic lobe in a Strong A mouse. Cells 3–5 layers deep, retaining their glandular activity, some showing early changes towards squamous epithelium. H. and E.

FIG. 7.—The same at a later stage. Cells acquiring more pronounced squamous metaplasia. H. and E.

FIG. 8.—The same after 3 weeks' time. Development of stratified squamous epithelium, 10 or more layers deep, keratin pearl formation still absent. H. and E.

PLATE II.

FIG. 9.—The same under higher magnification ; cells have broken through the basement membrane. H. and E.

FIG. 10.—The same at a later stage ; keratin pearl formation has taken place. H. and E.

FIG. 11.—Action of stilboestrol pellets (15 mg.) on the epithelium of the anterior prostatic lobe of a Strong A mouse. After 3 weeks' time, squamous metaplasia of glandular epithelium and formation of keratin pearls. H. and E.

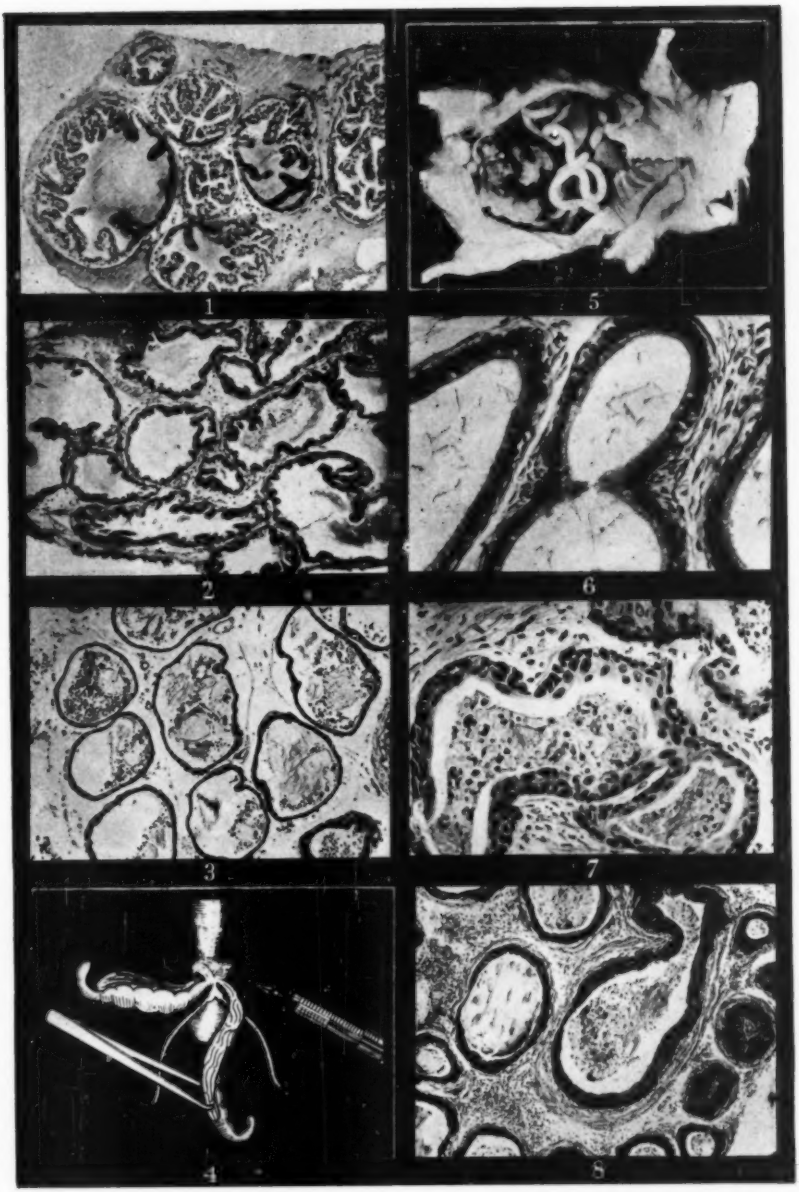
FIG. 12.—Squamous cell carcinoma with keratin formation of a prostate in a Strong A mouse after 5 months' treatment with methylcholanthrene. H. and E.

FIG. 13.—Similar type of tumour with alveolar structure partially preserved, three months' treatment with methylcholanthrene. H. and E.

FIG. 14.—A diffuse carcinoma in a Strong A mouse after 5 months' treatment with methylcholanthrene. H. and E.

FIG. 15.—Carcinoma with alveolar structure preserved and metaplastic cells ; 4½ months' treatment with methylcholanthrene. H. and E.

FIG. 16.—Sarcoma of the prostate in an R III mouse after 6 months' treatment with methylcholanthrene.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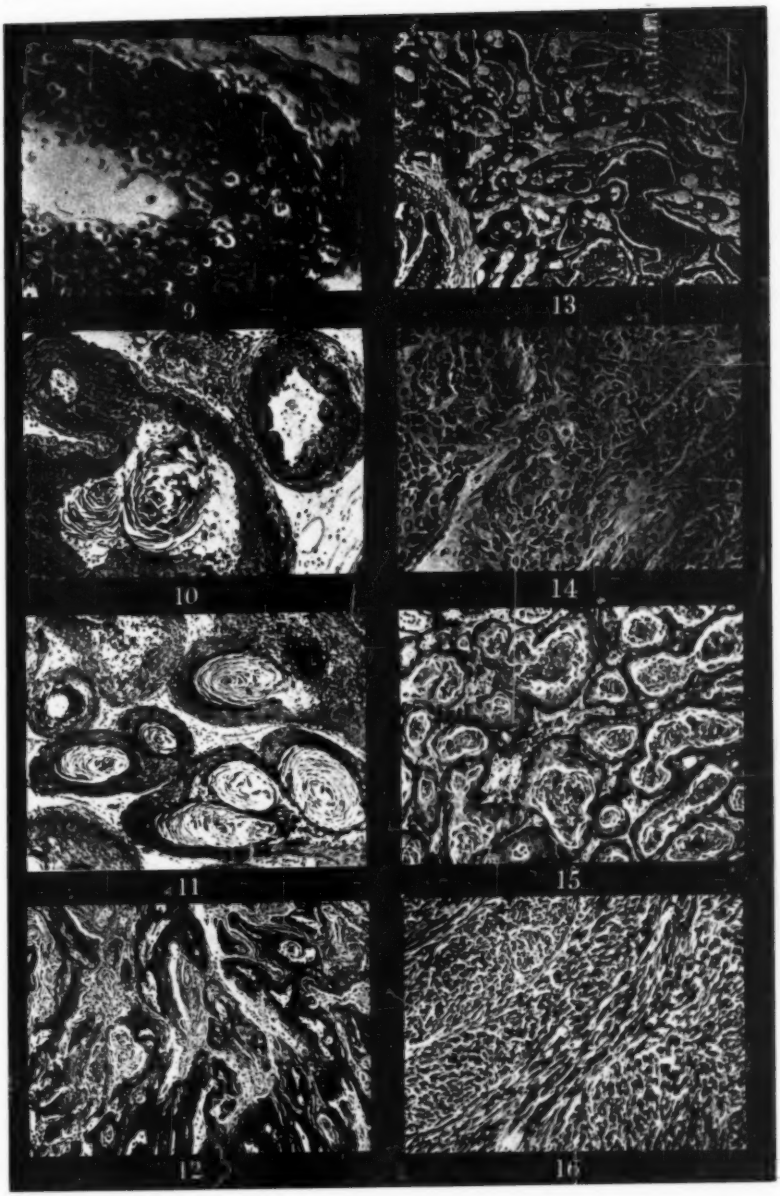
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of 20-methylcholanthrene in warm lard, was injected into either the dorsal or anterior prostates. Care was taken not to remove the needle of the syringe until the lard had set, in order to confine the carcinogens to the interior of the glandular tissues (Fig. 4).

The tumours were usually detected by palpation, or sometimes by the signs of distention of the bladder and retention of urine. Death from bilateral hydronephrosis occurred, however, in some mice before transplantation could be undertaken. The tumours which were transplanted grew successfully for many generations.

Primary and transplantable tumours were fixed in alcoholic Bouin, embedded in paraffin, and stained with haematoxylin and eosin, or by Masson's light green eosin technique.

RESULTS.

No detailed description of the microscopic appearance of the tumours need be given. They varied between $\frac{1}{2}$ to $\frac{3}{4}$ of an inch in diameter and frequently more than a single prostate lobe was involved.

A tumour of average size is shown in Fig. 5, in which it can be seen as a large central mass affecting all the prostatic lobes.

The number of mice used in the experiments and the incidence and types of tumours are shown in Table I.

TABLE I.

Strain of mice.	Number of mice.	Number of tumours.	Number of carcinomata.	Average time of appearance (months).	Number of sarcomata.	Average time of appearance (months).
Strong A	46 (8)*	22	10	4·0	12	4·8
R III	46 (14)*	17	..	..	17	7·0

* Numbers in brackets indicate the number of mice which died before the earliest tumour appeared.

As can be seen from Table I, both carcinomata and sarcomata of the prostate gland were induced in mice of Strong A strain, whilst in mice of R III strain, only sarcomata developed.

HISTOGENESIS.

Carcinoma.

Examination of the prostate following treatment with 20-methylcholanthrene showed the columnar epithelium undergoing squamous metaplasia. This change took place fairly rapidly in mice of the Strong A strain, leading to an epithelium 3–5 cells in depth. Some cells still retained their glandular activity (Fig. 6). Later phases of this metaplasia are shown in Fig. 7 and 8.

After 3 weeks the epithelium formed layers 5–10 cells thick with some of the basal cells infiltrating individually or in groups into the adjacent stroma (Fig. 9). At a still later stage keratin pearl formation occurred (Fig. 10).

It should be noted that the series of changes in the prostatic epithelium, following a single injection of methylcholanthrene are almost identical with those produced by prolonged treatment with stilboestrol in Strong A mice (Horning, 1946) (Fig. 11).

With oestrogen administration, however, the metaplasia and keratin pearl

formation is never followed by malignant changes which take place after treatment with 20-methylcholanthrene.

Fig. 13, 14 and 15 show the types of tumours induced.

DISCUSSION.

Moore and Melchionna (1937*a*, 1937*b*) induced tumours of the prostate gland in rats of unknown genetic constitution, by injecting 3:4-benzypyrene into the anterior prostate lobe. Recently Dunning, Curtis and Segaloff (1946) by implanting pellets of compressed methylcholanthrene crystals into the prostate gland of rats, induced squamous-celled carcinomas. One of these squamous tumours was found to be transplantable and regularly to form metastases in the lungs, lymph nodes and skeleton. The epithelial changes which followed were similar to those found in the present experiments with Strong A mice.

While in the rat the anterior lobes differ histologically (and also functionally) from the rest of the prostate (Moore, Price and Gallagher, 1930), in the mouse the anterior lobes are similar in structure to the dorsal lobes. In the present experiments, therefore, 20-methylcholanthrene was injected either into anterior or dorsal lobes, with similar results.

Moore and Melchionna (1937*a*, *b*) observed that sarcomata were induced in rat prostate glands in which carcinomata also developed; similar results were not obtained in these experiments.

There was a similar average time of appearance for both carcinomata and sarcomata in Strong A mice. In the R III strain, however, the tumours (sarcomata) developed at a later average age. The earliest tumours appeared after three months following the injection of methylcholanthrene in both strains of mice. Metastases were not observed, but the invasive growth into the surrounding tissues, and progressive growth after transplantation, leave no doubt that the tumours induced by 20-methylcholanthrene, were malignant.

In attempting to compare the tumours experimentally induced by carcinogens in mice and rats, with those arising spontaneously in man, it should be noted that the common form of carcinoma in man is an adeno-carcinoma. Carcinoma simplex is occasionally found, while sarcomata are rare in adult man, although they have been recorded in children (Fowler, 1942).

In rodents, spontaneous cancer of the prostate so far as we are aware is unknown; although some experimental tumours in mice and rats retained a glandular structure, there was generally a tendency to metaplasia and keratinization rather than a growth of ordinary adeno-carcinoma.

SUMMARY.

(1) Transplantable tumours of the prostate gland were induced in Strong A and R III mice following a single injection of 20-methylcholanthrene in lard into the anterior and dorsal lobe.

(2) Squamous metaplasia of the glandular epithelium occurred in both strains, but was followed by malignant proliferation of the metaplastic cells, only in the Strong A strain.

(3) In Strong A mice both carcinoma and sarcomata were found, whereas in R III mice only sarcomata developed.

(4) Carcinomata were of the squamous-cell type; sarcomata, in both strains, were of the spindle and polymorphic cell type.

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INDUCTION OF AN EXPERIMENTAL TUMOUR OF THE LENS.

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IT is a widespread doctrine among students of cancer that any tissue or organ capable of cell division may be the site of a malignant neoplasm, but it is well known to clinicians that certain tumours, theoretically possible, do not in fact occur. The most striking example of an organ which never gives rise to a neoplasm is the lens. Notwithstanding the fact that mitoses occur in the lens throughout life, having been seen even in the lenses of patients 80 years old, no tumour of the lens has ever been discovered.

The lens is an entirely epithelial structure embryologically derived from the surface ectoderm, devoid of blood supply and enclosed in a semi-permeable membrane, the hyaline lens capsule. Although mammalian lenses are enclosed during embryonic life in a capillary net (the vascular lens capsule) this does not constitute a blood supply, since the vessels do not come in contact with the lens cells, being always outside the hyaline capsule. In the majority of vertebrates even this embryonic vascular capsule is absent. The lens can therefore be looked on as a segregated group of specialized epithelial cells growing and differentiating in a closed system, separated alike from the blood-stream and from the surface of the body. The lens is composed of an anterior subcapsular epithelium (a single layer of cubical cells) in which the mitoses occur, and which, in the equatorial region, differentiates into the long lens fibres which are incapable of division.

The fibres in the centre of the lens lose water and shrink, and the size and shape of the lens throughout life is maintained by constant addition of new lens fibres differentiated from the subcapsular epithelium.

It thus appeared an interesting problem to discover whether the lens epithelial cells possess some intrinsic resistance to neoplastic change, or whether they owe their singular immunity to their position of anatomical isolation. Experiments were therefore devised and carried out which show that given certain conditions, the cells of the subcapsular epithelium are as able to produce cancer as are the epithelial cells of the skin.

The technique of the experiments was suggested by the work of Rous and Smith (1945), who showed that cancer could be induced by implantation of embryo skin and chemical carcinogens intramuscularly in adult animals, using inbred strains of mice. The C3H strain was chosen in the present experiments on account of the readiness with which tumours can be produced with methylcholanthrene in these mice. This was in some ways a disadvantage, since many tumours of the host mouse were induced and relatively few of the graft; but three undoubted cancers of lens epithelium have been obtained.

Experiments.—Thirty-eight experiments were performed and 26 of them resulted in tumours. Of these, three were carcinomas of the lens epithelium, and the remainder various types of sarcoma of the host mouse or epitheliomas of its skin. The three lens tumours occurred in the subcutaneous tissue of the flank of male mice, which rules out any possibility of derivation from mammary gland tissue of the host.

DESCRIPTION OF PLATES.

FIG. 1.—Antero-posterior section of embryo mouse lens.
a. Subcapsular epithelium.
b. Equatorial region showing transition into lens fibres.
c. Lens fibres.
d. Hyaline capsule.
e. Capillaries of vascular capsule.

FIG. 2.—Subcapsular epithelium and equatorial region of lens of 7-day-old mouse.
a. Region of mitoses.
b. Beginning differentiation of cells resulting from mitoses at *a*. The cells are lengthening and their nuclei are at the outer end.
c. Newly formed lens fibres.

FIG. 3.—Tangential section through the subcapsular epithelium of the mouse lens at the anterior pole.

FIG. 4.—Living tissue culture (2 days) of mouse lens (late embryo) showing ruptured hyaline capsule in centre, surrounded by swollen lens fibres, and at the outer edge, especially at "*a*," a sheet-like outgrowth of subcapsular epithelium.

FIG. 5.—Living tissue culture (2 days) of subcapsular epithelium of 9-day-old mouse.

FIG. 6.—Section through lens tumour.
a. Mitosis.
b.b. Nuclei showing typical arrangement of chromatin.

FIG. 7.—Smear preparation of lens tumour M8.134. The typical chromatin arrangement is seen at *a*. The displacement of the nucleus to one side of the cell can be recognized.

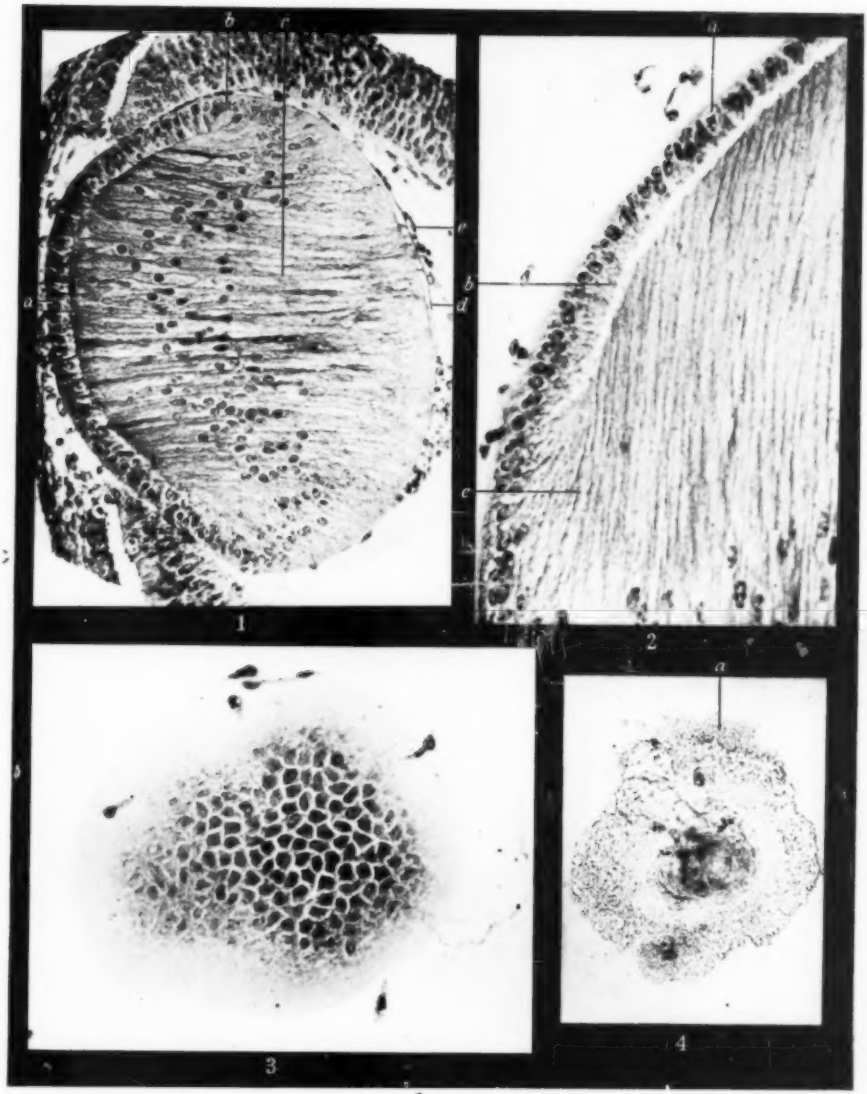
FIG. 8.—Living tissue culture of lens tumour at 48 hours.

FIG. 9.—Extending flat sheet of cells at edge of living tissue culture of lens tumour at 4 days.

FIG. 10.—Five-day living tissue culture of lens tumour M8.134. The explant is on the left, the new sheets of cells are breaking away on the right.

FIG. 11.—Fixed and stained preparation of 5-day tissue culture of tumour M8.134. Note character of nuclei and presence of mitotic figures.

FIG. 12.—Growing edge of 5-day tissue culture of lens tumour M8.134, showing fibrillar processes from the cells. The attempt at differentiation shown by the movement of the nucleus to the side of the cell is seen at *a.a*. Some of these displaced nuclei are pyknotic. Fixed preparation.



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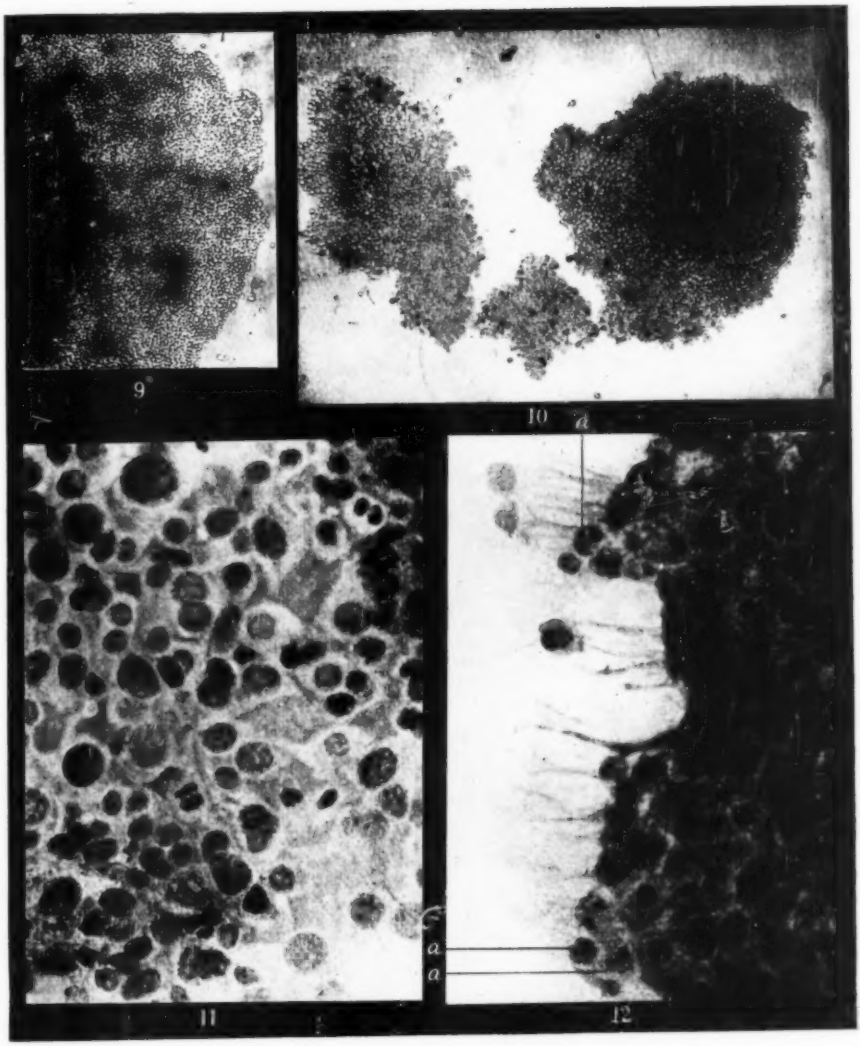
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Technique.—The lenses were obtained from the eyes of mice of various ages (new born, 2 days, 3 days, 4 days, 7 days and 9 days old), but tumours were induced only with 7-day and 9-day-old lenses. By careful dissection of the eyes it is possible to get the lenses in their hyaline capsules completely free from vascular capsule or uveal tissue. These lenses were inserted under the skin of the flank of mice of the same inbred strain together with methylcholanthrene. In some cases the hyaline capsules were ruptured and the lenses mixed with a few crystals of methylcholanthrene. In others the unruptured lenses coated with a solution of the carcinogen in oil were used. In others again teased lenses were mixed with methylcholanthrene in solution in equal parts of soft and liquid paraffin. The exact method employed did not appear to be important, two of the tumours being obtained with a solution of 1 mg. of methylcholanthrene per 0·5 c.c. of a mixture of soft and liquid paraffin and the third tumour with crystals only. In all the successful experiments the lens capsules were ruptured. The tumours appeared between two and three months after implantation.

Characteristics of tumours of lens epithelium.

The tumours are anaplastic carcinomas possessing certain characteristics suggestive of their origin from the subcapsular epithelium of the lens. A brief consideration of the normal structure and behaviour of this in mice is a necessary preliminary to the study of the tumours.

The normal lens and its epithelium is seen in Fig. 1, 2 and 3. Fig. 1 shows an antero-posterior section of the lens of a mouse embryo. The subcapsular epithelium is seen at *a*. Fig. 2 shows a portion of the subcapsular epithelium and the equatorial region of the lens of a 7-day-old mouse. The mitoses occur in the intermediate zone about "*a*," and the daughter cells differentiate into lens fibres as they are displaced backwards towards the equator. The first stage of this differentiation is the lengthening of the cell and the displacement of the nucleus to one end. Once this has occurred the cell cannot divide again, but proceeds to complete differentiation into a lens fibre, the nucleus of which eventually disappears. Fig. 3 shows a tangential section (at right angles to the plane of Fig. 2) passing exactly through the subcapsular epithelium at the anterior pole. The cells are cubical, with large nuclei, one or two prominent nucleoli and a stellate or reticular arrangement of chromatin (also seen in Fig. 2).

It has been shown by Kirby (1926) that attempts to grow the lens in tissue culture result in the production of flat sheets and detached islands of the undifferentiated subcapsular epithelial cells only. No growth occurs unless the hyaline capsule is ruptured and no lens fibres are ever formed, though some of his preparations show fibrillar prolongations of the cells. He used chick material only.

In view of the fact that the lens tumours grew fairly easily in tissue culture, an attempt was made to cultivate normal mouse lens *in vitro*. This proved difficult, but Fig. 4 and 5 show results at three and at two days. Fig. 4 is a tissue culture of the whole lens of a late mouse embryo. The ruptured capsule is in the centre of the explant and is surrounded by degenerating lens fibres. From the edges of the mass a sheet of epithelial cells is just beginning to grow out.

Fig. 5 is a surviving sheet of subcapsular epithelium in a 2-day tissue culture of a 9-day-old mouse lens. It demonstrates the arrangement in a flat pavement.

The three tumours obtained from the implantation of mouse lenses and methylcholanthrene show characteristics similar to each other and to the normal subcapsular epithelium. The first tumour obtained (known as M8.134) can be taken as the type and described in full.

*Protocol of Experiment M*8.134.—The lens of a 7-day-old mouse (C3H) was mixed with a 0·2 per cent solution of methylcholanthrene in equal parts of soft and liquid paraffin and inoculated into the subcutaneous tissues of the right flank of a male C3H mouse one month old. The small lump resulting from the inoculation remained for 3–4 weeks and finally disappeared. A tumour appeared at the site fairly suddenly about 2½ months after inoculation and grew rapidly, being ready for transplantation a fortnight later. The mouse was killed and the tumour examined and transplanted into six C3H mice. Transplants, tissue cultures, smear preparations and sections were made from the tumour and its subcultures. It has retained its characteristics through eight passages so far, and continues to grow rapidly, no failure being noted in any of the transplants.

*Characteristics of the lens tumour M*8.134.

The tumour tissue is greyish and translucent. Grafts grow rapidly, forming large fluctuating tumours containing a variable amount of necrotic tumour tissue and blood. Sections of the periphery of the tumour show the structure of an anaplastic carcinoma (Fig. 6). The cells are large, with voluminous spherical or oval nuclei, showing one or more nucleoli and a prominent reticular chromatin structure. Mitoses are frequent. In the young cells the nucleus is central, in the older cells it moves to the edge of the cell just before becoming pyknotic. Metastases occurring in the inguinal lymph nodes show the same structure, but with less necrosis. The tumour is soft, and the cells can be well seen in smear preparations (Fig. 7).

The tumour was grown in tissue culture* and Fig. 8, 9 and 10 show some results. Fig. 8 shows the explant after 48 hours. Tumour cells and a few fibroblasts are beginning to wander out. By four days (Fig. 9) the tumour cells have arranged themselves in the flat sheet typical of tissue cultures of carcinomas. This very much resembles the sheet of growing normal lens epithelium seen in Fig. 5. If the culture is continued the sheet of cells tends to break away from the tumour explant and large and small islands of cells float free in the medium. Fig. 10 shows this, and also demonstrates clearly the epithelial character of the cells. A high power photomicrograph of a portion of the sheet in Fig. 10 is seen in Fig. 11. The preparation is fixed and stained. Mitotic figures and completed mitoses are seen. The similarity of the cells to those of the tumour seen in Fig. 6 and 7 is apparent. In some of the tissue cultures of the tumour the extending edge showed fibrillar outgrowths somewhat similar to those described by Kirby in tissue cultures of chick lenses. These are seen in Fig. 12. This fixed and stained preparation of a 5-day tissue culture of the same tumour also

* The best medium was found to be one part of rat serum, two parts Tyrode solution, and one part mouse embryo extract. Sometimes a few drops of distilled water were added.

demonstrates the attempted differentiation by movement of the nucleus to the side of the cell before pyknosis sets in.

The other two tumours resembled M8.134 in their structure and behaviour. Both of them proved to be slower in growth. One appeared about a fortnight sooner and did not require transplanting for 4 weeks. The other tumour was intermediate in rate of growth. It shows the same characteristic epithelial cells, but also contains sarcomatous areas, probably derived from the host.

The main points which show these tumours to be derived from the implanted lens epithelium are their carcinomatous character, the basic similarity of the cells to lens epithelium in morphology, in their growth in tissue culture in friable sheets and in the displacement and degeneration of the nuclei in the course of attempted differentiation.

It is therefore certain that in given conditions the lens can undergo neoplastic change, and that its complete immunity in the body cannot be due to any inherent resistance in the cells. Its peculiarly isolated anatomical position and its complete lack of blood supply are more likely to account for its escape than are any peculiarities in the cells composing it or in its metabolism. Its structure indeed differs somewhat in the various species of vertebrates, but in every instance it is both ontogenically and phylogenetetically entirely avascular.

SUMMARY.

The statement has been made by general pathologists that any tissue or organ capable of cell division may be the site of a malignant neoplasm. Ophthalmologists, however, agree that carcinoma of the lens is unknown, although mitoses occur in the lens epithelium throughout life. The possible reasons for this are discussed. Experiments showing the induction of cancer of the lens in mice are described, using pure line strains and chemical carcinogens. These demonstrate the ability of the lens epithelium to become malignant under certain conditions, and throw some light on the immunity of the organ in the body.

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THE COMBINED ACTION OF 2-ACETYLAMINOFLUORENE AND SEX HORMONES IN THE WISTAR RAT.

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THE exceptional interest of 2-acetylaminofluorene (AAF) as a carcinogen was clearly revealed by Wilson, DeEds and Cox (1941), who fed it to inbred albino rats in which only benign mammary tumours had been observed, and found carcinomas of the liver, breast, bladder, ureter, renal pelvis, colon, pancreas, lung and skin, a myogenic sarcoma, and in two cases liver lesions resembling leukaemic infiltration. The results of Bielschowsky (1944 *a* and *b*) and other workers are summarized later in this paper. In CBA mice, neoplasms of liver and uterus (one a sarcoma) and of the urinary bladder were found by Armstrong and Bonser (1944). Bielschowsky and Green (1945) reported a carcinoma of the kidney in a Rhode Island Red cock which had been fed AAF for 45 weeks.

The widespread variety of organs affected by AAF led Bielschowsky (1944*b*) to try to induce tumours of the goitrous thyroid gland with this substance. Solid, parenchymatous goitre was induced by allyl thiourea; in conjunction with AAF, also orally administered, allyl-thiourea induced adenoma in 9 out of 10 rats, while malignancy was observed in 3 of these rats. Griesbach, Kennedy and Purves (1945) claim that the goitrogenic agent in *Brassica* seeds can cause adenoma of the thyroid if given orally for long enough, but only 1 of 8 Wistar rats developed such a lesion. Subsequently Purves and Griesbach (1946) obtained malignant changes in 2 out of 30 Wistar rats given thiourea in their drinking water for nearly two years. Purves and his co-workers consider that the effect of the goitrogens is indirect, operating by inducing hypersecretion of the thyrotropic pituitary hormone. They also suggest that the earlier appearance of malignancy in Bielschowsky's material is due to a synergistic action of AAF and the goitrogen employed, i.e. AAF has no specific action even on adenomatous thyroid tissue.

The success achieved by Bielschowsky in at least accelerating malignant changes in the thyroid of rats by feeding AAF suggested that other organs apparently untouched by AAF alone might undergo neoplastic changes when stimulated to proliferate by suitable means. The sex-organs of the rat can be caused to hypertrophy by the administration of the appropriate sex-hormone, and it was thought that these organs, thus caused to proliferate, might proceed to malignant changes when influenced by orally administered AAF. An exploratory experiment was therefore carried out and, although it failed in its primary objective, the recent publication of a paper by Cantarow, Paschkis, Stasney and Rothenberg (1946) reporting similar work in a different strain of rat makes it desirable to record the results obtained in this laboratory.

EXPERIMENTAL.

The rats used were of a Wistar strain inbred in this laboratory from rats originally bought from Glaxo, Ltd.; the ages varied from 5 to 22 weeks at the commencement of the experiment. The basal diet for all groups was rat-cake made to the formula of Thomson (1936). 2-Aminofluorene was synthesized from fluorene by the method of Diels (1901),* and acetylated as follows. Up to 1 per cent of concentrated sulphuric acid was added to 2-aminofluorene stirring in an excess of acetic anhydride which became very hot and dissolved the amine. After chilling thoroughly the acetylated amine was filtered on a sintered-glass funnel, and dissolved in hot ethanol. Sufficient water was added to cause permanent turbidity and the solution well cooled. The precipitate, after drying, had m.p. 193–194° C. It was recrystallized from methanol; m.p. 194° C.

AAF was administered in the rat-cake, which was supplied as a fine powder; assuming that a rat would eat 10 g. rat-cake powder per day, AAF was added at a level of 0·04 per cent, i.e. 4 mg. per rat per day.

Administration of sex-hormones.—Two sex-hormones were used, testosterone propionate obtained from Boots, Ltd., and oestradiol benzoate obtained from British Drug Houses, Ltd.

Mark and Biskind (1941) found that the subcutaneous implantation of pellets of testosterone propionate in normal rats led to enlargement of both prostates and seminal vesicles. Emmens (1941) found that 40 mg. pellets of disc-shape allowed an absorption of this hormone of 350γ per day. Forbes (1941), using 10 mg. pellets, found 90 per cent absorption in 61 days, i.e. an average of 150 γ per day. On the basis of these findings it was decided at first to implant one 10 mg. pellet in the flank of each rat every two months. However, in a private communication, Dr. C. W. Emmens pointed out that absorption during the second month would be much reduced, and recommended the use of 20 mg. pellets. Therefore thin disc-shaped pellets weighing 20 mg. were prepared and inserted subcutaneously (after incision and followed by two stitches) in alternate flanks, at intervals of two months. Pellets were completely absorbed by the time another was inserted in the same flank, i.e. by four months. The female hormone, oestradiol benzoate, is known to produce its action in lower concentrations than is required of the male hormone used; pellets were therefore prepared weighing 10 mg., and were inserted in other rats in the same way and at the same intervals.

Groupings.—A number of possibilities had to be covered in these experiments; in this way the following groups were determined:—

I. Sex-hormones alone:

(*a*) Testosterone propionate in virgin males.
Oestradiol benzoate in virgin females.

(*b*) Testosterone propionate in mated males.
Oestradiol benzoate in mated females.

II. Sex-hormones in conjunction with AAF:

(*a*) Testosterone propionate in virgin males.
Oestradiol benzoate in virgin females.

* I am indebted to Dr. E. de Barry Barnett for a gift of fluorene; later preparations have been made from fluorene obtained from British Drug Houses, Ltd.

(*b*) Testosterone propionate in mated males.
Oestradiol benzoate in mated females.
(*c*) Testosterone propionate in mated females.
Oestradiol benzoate in mated males.

The number of animals used for each group is recorded in Table I, which

TABLE I.—*Distribution of Rats by Cages.*

Cage No.	Testosterone propionate.	Testosterone propionate + AAF.*	Oestradiol benzoate.	Oestradiol benzoate + AAF.*	Totals. Male.	Totals. Female.
85	..	3 M.	..	..	3	..
86	3 M.	..	..	..	3	..
87	..	2 M. 2 F.	..	..	2	2
88	2 M.	..	2 F.	..	2	2
89	..	2 M.	..	2 F.	2	2
90	..	..	4 F.	..	..	4
91	..	..	..	3 F.	..	3
92	..	..	..	2 M. 2 F.	2	2
Totals	5 M.	7 M. 2 F.	6 F.	2 M. 7 F.	14	15

* AAF = 2-acetylaminofluorene.

also shows the distribution. Full data for individual rats are recorded in Table II.

The average weights of liver, prostates, testes + epididymis and seminal vesicles in normal adult males of our strain are as follows :—

Liver : 4 per cent of body weight.
Prostates : 0·15 per cent of body weight.
Testes + epididymis : 2 per cent of body weight.
Seminal vesicles : 0·3 per cent of body weight.

RESULTS.

The incidence of tumours in each group is summarized in Table III. A spindle-cell sarcoma of the left seminal vesicle was found in rat 354 (Group II*b*), and a whorled, spindle-cell sarcoma was found lying above the liver of rat 339 (Group II*a*). Tapeworms were not present in the liver of any rat.

DISCUSSION.

The results presented here agree with those of Cantarow *et al.* (1946) in that no carcinomas were induced in hypertrophied gonads in either male or female rats. The reason for this may well be that suggested by these workers, namely that " the hyperplasia of target organs of sex-hormones is a functioning hyperplasia, whereas that induced in the thyroid by goitrogenic agents is non-functioning." Thus the type of hyperplasia induced by Bielschowsky in the rat thyroid in which growth is accompanied by loss of differentiation would appear to have been a suitable basis for carcinogenesis, whereas the hyperplasia associated with increased differentiation induced in sex organs by over-stimulation with sex-hormones is apparently unsuitable. The reparative hyperplasia found in the livers of rats given N, *N*-dimethyl-*p*-aminoazobenzene (butter yellow) is often accompanied by primary liver-cell carcinoma, and it has been considered that the carcinoma arises in such nodules of regenerative tissue (Orr, 1940 ; see also

TABLE II.—*Data for Individual Rats.*

Group.	Rat No.	Sex.	Age at start, in weeks.	Nature of pellets inserted.	Pellets inserted on days :	Experimental period in days.	Weight at death. (g.)	§ Relative Weights (%) of			
								Prostates.	Testes + epididymis.	Seminal vesicles.	Liver.
I*a*	341	M.	9	20 mg. T.P.	1, 84, 149, 210, 273	K.‖ 370	255	0·5	0·8	..	4·5
	342	M.	6	,,	1, 84, 149, 210, 273	K. 370	290	0·4	0·8	0·6	5·3
	343	M.	6	,,	1, 62, 127, 188, 251	K. 348	270	0·5	0·9	0·7	4·8
	357	F.	14	10 mg. E.B.	85, 149	255	190	..	..	..	..
	358	F.	14	,,	85, 149, 210	350	135	..	..	..	..
	359	F.	14	,,	85, 149	K. 232	195	..	..	..	..
	360	F.	5	,,	1, 52, 113	272	130	..	..	..	..
I*b*	349	M.	10	20 mg. T.P.	1, 84, 149, 210, 273	K. 373	275	0·4	0·8	0·5	6·0
	350	M.	10	,,	1, 84, 149, 210, 273	K. 369	310	0·4	0·8	0·5	4·3
	351	F.	10*	10 mg. E.B.	85, 149	180	170	..	..	..	..
	352	F.	14	,,	85	Lost by cannibalism	..	..	..	..	..
II*a*	337	M.	14	20 mg. T.P.	1, 84, 149, 210, 273	K. 366	200	0·4	1·2	0·8	17·0
	338	M.	10	,,	1, 84, 149, 210, 273	K. 367	280	0·3	1·1	0·3	..
	339	M.	12	,,	1, 84, 149, 210, 273	K. 367	240	0·4	0·5	0·6	13·3
	361	F.	22	10 mg. E.B.	17, 81	180	170	..	..	..	..
	362	F.	22	,,	17, 81, 142	K. 301	145	..	..	..	..
	363	F.	12	,,	17, 81	K. 203	180	..	..	..	..
II*b*	345	M.	6	20 mg. T.P.	1, 62, 127, 188, 251	K. 319	225	..	..	..	..
	348	M.	12	,,	1, 84, 149, 210, 273	K. 372	235	0·4	0·7	0·4	19·0
	353	M.	9	,,	1, 84, 149, 210	K. 377	240	0·4	1·4	..	16·0
	354	M.	6	,,	1, 84, 149, 210, 273	377	250	..	..	..	14·6
	355	F.	9†	10 mg. E.B.	85	K. 141	155	..	..	..	..
	356	F.	14‡	,,	149	K. 378	180	..	..	..	18·4
	367	F.	5	,,	1, 52, 113	K. 238	165	..	..	..	..
	368	F.	5	,,	1, 52, 113	K. 238	140	..	..	..	..
II*c*	365	M.	5	10 mg. E.B.	1, 52, 113	K. 280	130	Not visible	0·2	0·15	11·1
	366	M.	5	,,	1, 52, 113	K. 276	115	..	..	0·2	7·3
	346	F.	14	5 mg. T.P. in oil	1, 15, 29, 43	K. 82	180	..	..	..	..
	347	F.	12	20 mg. T.P.	1, 84, 149, 210, 273	K. 372	210	..	..	..	16·0

T.P. Testosterone propionate.
E.B. Oestradiol benzoate.
* Litter at 18 weeks.
† Litter at 20 weeks.
‡ Litters at 26 and 38 weeks.
§ Expressed as percentage of total weight at death.
‖ K. = killed.

TABLE III.—*Summary of Tumours Found in Each Group.*

Group.	Number of rats. M.	F.	Site. Liver.	Mammary glands.	Bladder.	Lung.	Stomach.
I*a*	3	..	None	None	None	None	None
		4	„	1 mixed tumour*	„	1 undifferentiated bronchial carcinoma	„
I*b*	2	2	„	None	„	None	„
II*a*	3	..	Hepatomas in all 3	„	„	„	„
		3†	Hepatomas in both	1, undifferentiated adenocarcinoma in right caudal gland	„	„	1, ulceropapillomas in fore-stomach
II*b*	4	..	3, hepatomas; 1, carcinoma; 1, cholangiomas	None	„	„	None
		4‡	2, hepatomas and cholangiomas	1, undifferentiated adenocarcinoma in left axillary gland	„	„	1, ulceropapillomas in fore-stomach
II*c*	2	..	Hepatomas in both	1, lactation in two lobes	1, squamous carcinoma	„	None
		1§	Hepatoma and cholangioma	None	None	„	„

* Adenoacanthoma and intra-canalicular adenomas.
† 1 died at 180 days with advanced post-mortem changes but no visible tumours.
‡ 1 killed at 141 days showing regenerative hyperplasia of liver.
§ 1 killed at 82 days showing no abnormality.

Opie, 1944). If this sequence is true, then the hyperplastic liver tissue may be more susceptible than resting liver tissue to the carcinogenic action of azo dyes. Preliminary work reported here suggests that the initial stage of reaction to AAF is not degenerative, but that the hyperplasia is induced by the compound directly.

A summary of results obtained by workers with AAF in rats is provided in Table IV. From this it is evident that AAF can induce tumours, usually malignant, in an even greater variety of sites than was apparent from the work of Wilson *et al.* (1941). Wilson *et al.* illustrate a "carcinoma arising adjacent to the external auditory canal," and discuss the histogenesis of this and other tumours in this locality included by them under "subcutaneous tumours." Bielschowsky first drew attention to tumours of the ductus acusticus externus as a separate entity. No such tumours were seen in any of the rats in the experiments described in this paper, although 9 survived more than 300 days' administration of AAF. As Wistar rats were also used by Bielschowsky, who found these tumours in 18/93 rats given AAF orally up to 210 days, the influence of diet seems to have some importance. When allyl-thiourea was also given, the incidence of tumours rose to 6/10. Lopez (1945) also found external meatus tumours in 2/4 rats. On the other hand, Heiman and Meisel (1946) make no mention of such tumours among 39 Wistar rats given AAF orally for up to 227 days. Bielschowsky (1944*a*) seems to have used a simpler and, perhaps, less adequate diet than did Heiman and Meisel; the basal diet used in our experiments was also better balanced than that of Bielschowsky. It has been reported (Harris, 1946) that protection against liver tumours due to AAF could not be obtained by adding to the basal diet supplements of foodstuffs known to afford considerable protection to the rat liver against carcinogenesis by azo-dyes. This further supports the apparent difference in carcinogenesis between azo-dyes and AAF, the former being dependent on reparative hyperplasia as a precancerous stage. It is significant, however, that Heiman and Meisel (1946) found that Wistar rats fed AAF plus aromatic amino-acids were free from liver tumours and cirrhosis up to 227 days. This may indicate that, in the rat liver, the carcinogenic process in the case of AAF depends on the "blocking" of a normal metabolic mechanism dependent on adequate supplies of aromatic amino-acids rather than on sulphur-amino-acids and/or riboflavin, which appear to be needed in excess of normal requirements to prevent (or delay) carcinogenesis of the liver due to azo-dyes.

Other sites in the Wistar rat in which Bielschowsky found tumours following administration of AAF include the small intestine (5/93 cases); Heiman and Meisel report no tumours in this site, and none was found in our experiments. Moreover, Bielschowsky reports mammary tumours in 27/93 Wistar rats, including 3 males; Heiman and Meisel found them in only 2/39 rats. It has been shown by Mark and Biskind (1941) that adeno-carcinoma of the breast in rats may follow the implantation of oestradiol benzoate pellets without administration of any other carcinogen. In our series, 1 of 3 control rats receiving oestradiol benzoate and 2/8 females receiving AAF as well developed mammary tumours; 1 male in Group IIc (oestradiol benzoate pellets) had lactating glands but no tumours. Hence the action of AAF on proliferating mammary tissue was only slight in our experiments.

In the matter of liver tumours, our series has yielded results in full accordance

TABLE IV.

Author(s).	Rat type.	Number of rats.	Periods up to—	Level AAF.	Tumour sites. Liver. M.	Liver. F.	Mammary tissue. M.	Mammary tissue. F.	Bladder. M.	Bladder. F.	External ear. M.	External ear. F.	Small intestine. M.	Small intestine. F.	Large intestine. M.	Large intestine. F.
Wilson *et al.* (1941)	Inbred albino	39	95 days or more	0·031%	8/9		1/9		4/9		..	..	..	..	..	..
				0·062%	5/5		1/5		2/5		..	..	..	..	..	..
				0·125%	23/25		1/25		19/25		..	..	..	..	1/25	
Bielschowsky (1944*a*)	Wistar	104	210 days	0·04%	39/46	16/47	3/46	24/47	..	..	10/46	8/47	3/46	2/47	..	..
Bielschowsky (1944*b*)	„	10	210 „	0·04% + 0·06% allyl-thiourea	4/5	1/5	0/5	2/5	..	..	2/5	4/5	..	..	..	..
Lopez (1945)	?	4	168 „	0·05%	2/4		2/4		..	..	2/4		..	..	..	..
Bielschowsky (1946)	Piebald	38	293 „	0·04%	10/13	10/25	..	1/25	..	..	10/13	20/25	6/13	14/25	..	2/25
Cantarow *et al.* (1946)	Sherman	21 controls	316 „	0·03%	2/3	4/18	..	..	..	..	..	..	..	..	..	..
		15	316 „	0·03% + 0·05% thiouracil	0/6	0/9	..	..	..	..	..	..	..	..	..	..
		25 F. oestrogen	300 „	0·03%	..	13/25	..	..	..	..	..	..	..	..	..	..
		15 M. androgen	302 „	0·03%	13/15	..	..	..	..	..	..	..	..	..	..	..
Heiman and Meisel (1946)	Wistar	39	227 „	5 mg. per day	1/39		2/39		..	..	..	..	..	..	..	..
Kirby	„	17	378 „	0·04%	9/9	5/8	0/9	2/8	1/9	0/8	0/9	0/8	0/9	0/8	0/9	0/8

TABLE IV (continued).

Author(s).	Rat type.	Number of rats.	Periods up to—	Level AAF.	Tumour sites. Skin. M.	Skin. F.	Uterus. M.	Uterus. F.	Ureter. M.	Ureter. F.	Kidney. M.	Kidney. F.	Lung. M.	Lung. F.	Brain. M.	Brain. F.
Wilson *et al.* (1941)	Inbred albino	39	95 days or more	0·031%	4/9	..	..	..	..	..	5/9	..	3/9	..	..	..
				0·062%	0/5	..	..	..	..	..	0/5	..	1/5	..	..	..
				0·125%	4/5	..	..	..	1/25	..	13/25	..	9/25	..	..	..
Bielschowsky (1944*a*)	Wistar	104	210 days	0·04%	..	1/47	..	1/47	..	..	..	..	..	..	..	..
Bielschowsky (1944*b*)	„	10	210 „	0·04%+ 0·06% allyl-thiourea	..	..	..	..	..	..	1/5	0/5	..	..	..	..
Lopez (1945)	?	4	168 „	0·05%	..	..	..	..	..	..	..	..	..	..	1/4	
Bielschowsky (1946)	Piebald	38	293 „	0·04%	..	..	..	..	..	..	..	..	1/13	2/25	..	..
Cantarow *et al.* (1946)	Sherman	21 controls	316 „	0·03%	..	..	..	..	..	..	..	..	..	..	..	..
		15	316 „	0·03%+ 0·05% thiouracil	..	..	..	..	..	..	..	..	..	..	..	..
		25 F. oestrogen	300 „	0·03%	..	..	..	..	..	..	..	..	..	..	..	..
		15 M. androgen	302 „	0·03%	..	..	..	..	..	..	..	..	..	..	..	..
Heiman and Meisel (1946)	Wistar	39	227 „	5 mg. per day	..	..	..	..	..	..	..	..	..	..	..	..
Kirby	„	17	378 „	0·04%	0/9	0/8	0/9	0/8	0/9	0/8	0/9	0/8	None	None	None	None

TABLE IV (continued).

Author(s).	Rat type.	Number of rats.	Periods up to—	Level AAF.	Tumour sites. Sarcoma. M.	Sarcoma. F.	Pancreas. M.	Pancreas. F.	Sub-maxillary gland.	Thyroid.	Para-thyroid.
Wilson *et al.* (1941)	Inbred albino	39	95 days or more	0·031%	..	..	5/9		..	..	..
				0·062%	..	..	2/5		..	..	..
				0·125%	1/25		11/25		..	..	..
Bielschowsky (1944*a*)	Wistar	104	210 days	0·04%	..	..	..	..	..	..	..
Bielschowsky (1944*b*)	„	10	210 „	0·04%+ 0·06% allyl-thiourea	..	..	..	..	..	9/10	..
Lopez (1945)	?	4	168 „	0·05%	..	..	..	..	..	..	..
Bielschowsky (1946)	Piebald	38	293 „	0·04%	..	..	..	..	..	..	..
Cantarow *et al.* (1946)	Sherman	21 controls	316 „	0·03%	..	..	..	..	..	..	..
		15	316 „	0·03%+ 0·05% thiouracil	..	..	..	..	..	..	..
		25 F. oestrogen	300 „	0·03%	..	..	..	..	..	..	..
		15 M. androgen	302 „	0·03%	..	..	..	..	..	..	..
Heiman and Meisel (1946)	Wistar	39	227 „	5 mg. per day	1/39		..	..	5/39	1/39	2/39
Kirby	„	17	378 „	0·04%	2/9	0/8	None	None	None	None	None

with those of Bielschowsky. All of 9 male rats, dying between 276 and 377 days, had liver tumours; most had some degree of cirrhosis, and cholangiectasis, in varying degree, was frequently found. One rat, in Group II*b*, showed lesions varying from fatty degeneration to acute yellow atrophy; cholangioma with haemorrhage into cystic ducts was also present. The other 8 had either diffuse or trabecular hepatoma; bile-duct proliferation was also seen in these rats. Of the 8 female rats examined, 2 died at 82 and 141 days respectively without tumours; of the remaining 6, 5 showed hepatoma and 3 cholangioma. Cholangiectasis was present in most, but cirrhosis in only 1 case.

Cantarow *et al.* (1946) remark that "the occurrence of the cystic and neoplastic lesions was not paralleled by degenerative or fibrotic processes in the liver," and this was certainly true of the livers in our series.

Cantarow *et al.* (1946) only report on the liver lesions induced in Sherman rats by orally ingested AAF. Here again, however, it is clear that the incidence is lower in females than in males. The types of liver tumours seem to be essentially the same as in Wistar rats. This is also true of the liver tumours obtained by Bielschowsky in piebald rats, in which the sex-ratio is also maintained and the incidence much the same. In the Sherman strain, Cantarow *et al.* found the incidence of liver tumours in female rats raised when oestradiol dipropionate was injected intramuscularly; but the complete absence of liver neoplasms in rats given thiouracil was much more striking. The latter group ate less of the AAF diet than did controls receiving no thiouracil, but more than the oestrogenized rats.

The incidence of mammary tumours in piebald rats was very low compared with that in Wistar rats on the same diet (Bielschowsky, 1946). But the incidence of tumours of the ductus acousticus externus was much higher in the piebald rats, in which tumours of the small intestine were also much more frequent. Moreover, lung tumours were found in this strain, but have not been reported in Wistar rats given AAF and were not found in our series, although one control female (Group I*a*) was found to have undifferentiated bronchial carcinoma.

The albino rats of Wilson *et al.* (1941) resemble the piebald strain in the low incidence of mammary tumours, but differ in the absence of tumours of the small intestine. On the other hand, lung and liver tumours were rather more frequent. Moreover, Wilson *et al.* found tumours in the urinary system (kidney, ureter and bladder) and in the pancreas. The latter have not been found in any other strain of rat, and tumours of the kidney and bladder only once each.

It would seem that, if sufficient strains of rat were employed, possibly no site in the body would be found immune from the carcinogenic action of AAF. In the strains so far tested, only mammary tissue among the sex-organs seems to be susceptible, and this tissue is liable to develop carcinoma due to the action of oestrogens alone. Sarcomas are rare, and are not induced by subcutaneous injection of AAF, at least in Wistar rats. In our series, a whorled, spindle-cell sarcoma was found in the omentum covering the left lateral lobe of the liver in one rat, and a spindle-cell sarcoma was present in the left seminal vesicle of another.

It remains to discuss a few other points of interest. Toxic damage to kidneys was seen in some animals of all groups; rats receiving oestradiol benzoate pellets, with or without AAF, tended to show degeneration of the epithelium of the first and second convoluted tubules with the presence of a dark yellow

pigment in these cells. There was no consistent evidence of toxic damage to the kidneys by AAF or its metabolites, nor were any tumours of this organ seen.

No tumours of the uterus were found, but all females receiving oestradiol benzoate had pyometra, which often made it necessary to kill the animal; the 2 females receiving the male sex-hormone had no pyometra.

The spleens of the control groups, I*a* and I*b*, showed no abnormality. But 1 female in Group II*a* and 1 male and 2 females in Group II*b* had enlarged spleens, due to lymphoid hyperplasia. There was no question, however, of the regular, early, gross enlargement of the spleen seen in rats ingesting azo-compounds (Smith, Lillie and Stohlman, 1943; Kirby and Peacock, in press). It may be concluded that no splenotoxic substance passes from the liver into the blood of rats fed AAF; rats fed *p*-aminoazobenzene (AAB) or its N-monomethyl- or N, N-dimethyl-derivatives (MAB and DAB) have AAB circulating in the blood and affecting the spleen (Miller, Miller and Baumann, 1945).

The rats given AAF orally in the experiments described in this paper developed tumours of the liver predominantly in the right lobes; tumours in the left side of the liver were usually smaller or absent. Hahn, Donald and Grier (1945) found that injections of phosphate (tagged with radio-active phosphorus) into the mesenteric vein in dogs led to a large excess of isotope in the right side of the liver; similar injection of the splenic vein resulted in excess isotope in the left side. Shaffer, Carpenter and Moses (1946) have found that carbon tetrachloride injected into the mesenteric vein in dogs damages the right side of the liver rather than the left. Hence AAF would appear to reach the liver *via* the mesenteric "flow" and so cause pathological changes in the right side first; the appearance of tumours in the median and left lobes later, and also in many other sites in the rat body, suggests that either the metabolism of AAF in the liver is not efficient (i.e. AAF leaves the liver as such), or that a carcinogenic metabolite passes into the systemic circulation. A similar predominance of tumours in the right side of the rat liver has been reported by Opie (1944) in rats fed DAB in diets not inducing cirrhosis. But Kirby and Peacock (in press) found tumours in the left side of the liver in rats given AAB orally for a long period, which suggests carcinogenesis following the continual inflow of AAB *via* the splenic vein.

SUMMARY.

1. 2-Acetylaminofluorene has been given orally to Wistar rats, of both sexes, in which pellets of sex-hormones were also implanted subcutaneously. No tumours in the target organs (gonads) were found which could be attributed to the action of the carcinogen.

2. Hepatoma, cholangioma and primary liver-cell carcinoma were found in these rats. Cirrhosis was not a constant finding. Female rats were less susceptible to tumour induction or cirrhosis than were males.

3. Tumours of the ductus acusticus externus, small intestine or lung were not found even in rats surviving more than 300 days' AAF feeding. Mammary tumours were found in two rats and a bladder carcinoma in one rat. Two sarcomas were found in unusual sites.

4. Liver tumours were mainly in the right (posterior and anterior) lobes and only later in the median and left lobes, never in the caudate lobe. The signifi-

cance of this and of the distribution of liver tumours caused by ingestion of azo-dyes is discussed.

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THE ABSENCE OF A DIRECT RELATION BETWEEN GROWTH INHIBITION AND SULPHUR METABOLISM AFTER 1:2:5:6-DIBENZANTHRACENE.

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HADDOW, Scott and Scott (1937) found that certain carcinogenic hydrocarbons, particularly 1:2:5:6-dibenzanthracene, produced immediate, constant and long-continued reduction in the growth rate of young rats, while some non-carcinogenic hydrocarbons did not show this effect. 1:2:5:6-Dibenzanthracene has also been found by Haddow (1935), and Haddow and Robinson (1937), to have a considerable inhibitory action on tumour growth. White and White (1939, 1940) have attempted to explain the inhibitory action on body growth of these hydrocarbons by the deprivation of essential sulphur-containing amino acids brought about by their combination with the hydrocarbon in detoxication mechanisms involving the formation of mercapturic acids. This explanation has been widely quoted in the literature and appears to have been fairly generally accepted, but in an investigation by Elson, Goulden and Warren (1945) no evidence of the excretion of mercapturic acids by rats treated with 1:2:5:6-dibenzanthracene or 3:4-benzpyrene was found, and a preliminary re-examination of the data of White and his collaborators indicated that their conclusions are not unequivocal, and that the growth-inhibitory action of these hydrocarbons cannot be attributed to any direct connection with sulphur metabolism.

Further evidence in support of this view has now been obtained in the course of an extended investigation of the growth-inhibitory action of 1:2:5:6-dibenzanthracene.

EXPERIMENTAL.

The conclusions of White and collaborators were based on the restoration of growth observed on addition of cystine or methionine to the diet of rats already retarded in growth by the inclusion of hydrocarbons in their food. Rats on the supplemented diet but still ingesting hydrocarbon showed an increased growth rate over that of those on the unsupplemented diet. This increase restored their growth rate to about the same level as that obtaining when they were fed the unsupplemented diet free from hydrocarbon.

Diets.

In the present experiments attempts have been made to reproduce as nearly as possible the diets used by White and White (1939).

Yeast is a constituent of these diets, and as no specific reference to dried yeast was made in their paper, it was first assumed that moist yeast had been

used, and an equivalent amount (1 per cent) of dried yeast was used in our basal diet.

A group of 18 rats (average weight about 50 g.) was maintained on this basal diet and weighed collectively every few days. The rate of growth of these rats was extremely slow, the average rate over 33 days being only 0·1 g./rat/day. As this was much below the growth rates reported by White and White it was assumed that dried yeast had probably been used in their diet. The yeast content of the diet was therefore adjusted to 5 per cent, the composition of the basal diet being now as in Table I. The change to this diet resulted in an

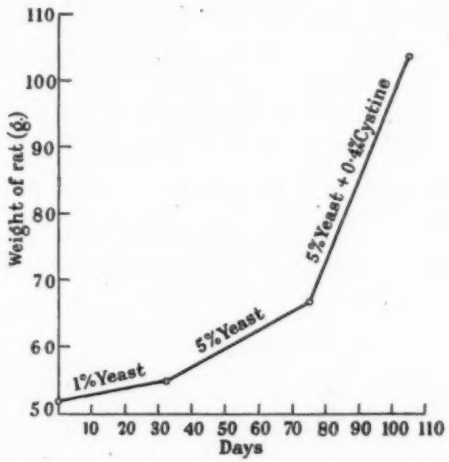

FIG. 1.—Average growth rate of rats maintained on " White " basal diet with 1 per cent and 5 per cent dried yeast, and with 5 per cent dried yeast + 0·4 per cent cystine.

TABLE I.—*Growth Rates of Rats on Basal Diet, and with Supplements of Cystine or Methionine.*

Rat No.	Basal diet.*		Basal diet + cystine (0·4 per cent).		Basal diet + methionine (0·5 per cent).	
	Total weight increase (26 days).	Increase in weight per rat/day.	Total weight increase (26 days).	Increase in weight per rat/day.	Total weight increase (26 days).	Increase in weight per rat/day.
1	21 g.	0·8 g.	31 g.	1·2 g.	38 g.	1·5 g.
2	22 g.	0·85 g.	32 g.	1·2 g.	37 g.	1·4 g.
3	28 g.	1·1 g.	18 g.	0·7 g.	35 g.	1·9 g.
4	20 g.	0·8 g.	20 g.	0·8 g.	51 g.	2·0 g.
5	22 g.	0·85 g.	38 g.	1·5 g.	37 g.	1·4 g.
6	...	...	28 g.	1·1 g.	...	...
7	...	...	37 g.	1·4 g.	...	...
8	...	...	54 g.	2·1 g.	...	...
9	...	...	48 g.	1·8 g.	...	...
Mean	23 g.	0·9 g.	34 g.	1·3 g.	40 g.	1·6 g.

* Composition of Basal Diet: Starch (Maize) 48 per cent, Lard 23 per cent, Sugar 14 per cent, Casein 6 per cent, Salt mixture (Glaxo) 3·0 per cent, Yeast (dry) 5·0 per cent, Cod liver oil 1 per cent.

increase in growth rate to an average of 0·3 g./rat/day over the next 42 days. 0·4 per cent of cystine was now added to the diet and a considerable increase in growth rate resulted immediately (average 1·2 g./rat/day over 30 days). (Fig. 1.)

The growth rates of individual rats maintained on the basal diet, basal diet supplemented with 0·4 per cent cystine and basal diet supplemented with 0·5 per cent methionine were then studied. The rats were kept in separate cages and weighed every two or three days and supplied with diet and water *ad libitum*. The results are given in Table I.

The addition of cystine to the diet results in an increase in growth rate from an average of 0·9 g./rat/day to 1·3 g./rat/day, whilst addition of methionine results in a greater increase in growth rate to 1·6 g./rat/day.

Production of fatty livers with 1:2:5:6-*dibenzanthracene on low protein diets.*

The White basal diet is a very poor diet for maintaining the health of the animals and a number of deaths occurred after a few weeks. The survivors, when subsequently killed, showed marked fatty infiltration of the liver. The addition of methionine to the basal diet affords good protection against fatty livers and the health of the animals was much improved. Cystine is not as reliable as methionine in this respect, probably on account of its toxic action (Curtis and Newburgh, 1927), and the growth rates of the animals in the cystine-supplemented diet show considerably more variation than those of the animals on the basal and methionine-supplemented diets.

In experiments on the growth-inhibitory action of 1:2:5:6-dibenzanthracene in rats maintained on a 5 per cent protein diet it was noticed that the treated animals had fatty livers. This 5 per cent protein diet (for composition see Table II) contained considerably less fat than the White basal diet, and the control animals maintained on it did not develop fatty livers during the course of the experiment (up to 56 days).

Although combination of the hydrocarbon with essential sulphur-containing amino acids has been shown not to be a direct cause of its growth-inhibitory action, it was considered possible that on the very low (5 per cent) protein diet such a combination might be sufficient to prevent the lipotropic action of these amino-acids, and thus result in the development of fatty livers. The fatty infiltration should, if this is the case, be prevented by methionine and possibly by cystine.

In order to test this, groups of rats were maintained (1) on the 5 per cent protein diet, (2) on this diet supplemented with 0·4 per cent cystine, and (3) on the diet supplemented with 0·5 per cent methionine. After a period of not less than 15 days from the start of feeding the diet, half the number of animals in each group were each given 50 mg. 1:2:5:6-Dibenzanthracene (suspended in 1 c.c. arachis oil) by intraperitoneal injection, and the remainder of the group left as controls. After a further period of at least 25 days the rats were killed and the fat content of the livers determined by the following method :

About 3 g. of liver was weighed out accurately and ground up in a mortar with about 20 g. of anhydrous sodium sulphate, and then transferred to an extraction thimble and extracted with ether for about 20 hours in a Soxhlet apparatus. The ether extract was transferred to a weighed evaporating dish, the ether evaporated and the residue weighed after drying for 15 minutes at 110°. The results are given in Table II.

TABLE II.—*Fat Content of Livers of Control and* 1:2:5:6-*Dibenzanthracene treated Rats Maintained on a 5 per cent Protein Diet.*

5 per cent protein diet.					5 per cent protein +0·4 per cent cystine.					5 per cent protein + 0·5 per cent methionine.				
Control.		1 : 2 : 5 : 6-dibenzanthracene treated.			Control.		1 : 2 : 5 : 6-dibenzanthracene treated.			Control.		1 : 2 : 5 : 6-dibenzanthracene treated.		
Number of days on diet.	Per cent fat.	Number of days on diet.	Number of days after DBA.	Per cent fat.	Number of days on diet.	Per cent fat.	Number of days on diet.	Number of days after DBA.	Per cent fat.	Number of days on diet.	Per cent fat.	Number of days on diet.	Number of days after DBA.	Per cent fat.
51	3·4	50	32	14·2	39	4·9	43	28	7·6	57	6·4	55	36	5·6
52	3·9	53	35	10·1	42	5·7	50	35	7·7	60	2·7	56	37	7·0
53	4·2	55	37	12·1	43	4·6	51	36	11·0	62	4·3	61	42	5·4
54	4·1	65	40	8·9	46	10·4	52	37	6·5	64	3·4	63	44	6·3
56	3·6	68	43	9·6	...	...	...	...	...	...	...	...	...	...
Mean	4·0			11·0		6·4			8·2		4·2			6·1

Composition of Diet: Starch 85 per cent, Casein 5 per cent, Bemax (Vitamins Ltd.) 2·5 per cent, Margarine 5 per cent, Calcium carbonate 0·5 per cent, Salt mixture (Glaxo Laboratories Ltd.) 1 per cent, Cod liver oil 1 per cent.

The weights of the rats were recorded every 2 or 3 days before and after the dibenzanthracene injection, and the growth rates thus obtained are given in Table III.

TABLE III.—*Growth Rate of Rats Treated with* 1:2:5:6-*Dibenzanthracene while Maintained on 5 per cent Protein Diets.*

5 per cent protein diet.		5 per cent protein + 0·4 per cent cystine.		5 per cent protein + 0·5 per cent methionine.	
Control period 14 days growth rate/ rat/day.	After DBA treatment 14 days growth rate/ rat/day.	Control period 14 days growth rate/ rat/day.	After DBA treatment 14 days growth rate/ rat/day.	Control period 14 days growth rate/ rat/day.	After DBA treatment 14 days growth rate/ rat/day.
1·0 g.	0·0	0·70 g.	0·30	0·64 g.	0·50 g.
0·9 g.	0·1	0·79 g.	0·0	1·30 g.	0·57 g.
0·93 g.	0·1	0·64 g.	0·0	1·10 g.	0·35 g.
1·30 g.	0·8	1·30 g.	0·45	0·80 g.	0·14 g.
1·35 g.	0·1	...	...	...	...
Mean 1·1 g.	0·22 Inhibition 80 per cent	0·86 g.	0·2 Inhibition 78 per cent	0·96 g.	0·39 g. Inhibition 60 per cent

The addition of cystine to the diet had a partial protective action against the development of fatty infiltration of the liver after administration of 1:2:5:6-dibenzanthracene, but had no effect in protecting the animal from the growth inhibition induced by the hydrocarbon. Methionine added to the diet afforded very good protection against fatty infiltration of the liver, but showed only a slight effect in counteracting the growth-inhibitory action of dibenzanthracene.

DISCUSSION.

The conclusion of White and collaborators that the growth inhibition produced by carcinogenic hydrocarbons is caused by their combination with essential sulphur-containing amino acids was based on the restoration of growth rate observed on addition of cystine or methionine to the diet of rats already retarded in growth by the inclusion of hydrocarbons in their food. Rats on the supplemented diet, but still ingesting hydrocarbon, showed an increased growth rate over that of those on the unsupplemented diet. This increase restored their growth rate to about the same level as that obtained when they were fed the unsupplemented diet free from hydrocarbon. In the present investigation it has been found that the addition of cystine or methionine to the basal diet without incorporation of the growth inhibitory hydrocarbon brings about an increase in growth rate of the animal; hence if the effect of this addition is also to negative the growth-inhibiting action of added hydrocarbon, the resultant growth rate should be greater than that produced by the basal diet alone, which, on White's results, is not the case.

The White basal diet is a very poor diet for maintaining the health of the animals, and a number of deaths occurred after a few weeks. The survivors when subsequently killed showed marked fatty infiltration of the liver. Protection against fatty liver development is afforded by methionine and to some extent by cystine, and the health of the animals is much improved by addition of these supplements to their diet.

It is not therefore surprising that addition of hydrocarbons to the White basal diet results in an inhibition of the already poor rate of growth of the

animals, and it is noticeable that almost every substance tested by White and collaborators—hydrocarbons, both carcinogenic and non-carcinogenic, azo compounds, and even aniline—produce growth inhibition, and that this inhibition is apparently restored by the addition of cystine or methionine to the diet.

In the present work addition of cystine to a 5 per cent protein diet and to a 10 per cent protein diet (Elson and Warren, 1947) failed completely to prevent the growth inhibition produced by injection of 1:2:5:6-dibenzanthracene. It must be concluded therefore that there is no direct relation between sulphur metabolism and the growth inhibition produced by 1:2:5:6-dibenzanthracene. This, however, does not mean that combination of the hydrocarbon with sulphydryl-containing substances does not occur. It appears very probable that such a combination takes place as one of the first steps in the metabolism of carcinogenic hydrocarbons and, indeed, of many aromatic compounds. The fatty infiltration of the liver produced by 1:2:5:6-dibenzanthracene in rats maintained on a 5 per cent protein diet is most probably caused by a reaction of this kind, since it is prevented by addition of methionine or cystine to the diet. That such a reaction of a carcinogenic substance with sulphydryl groups plays a part in carcinogenesis is suggested by Crabtree (1946*a*, 1946*b*), who has shown that disturbance of sulphur metabolism impairs carcinogenic activity, and concludes that the hypothesis that a primary action of carcinogens is their fixation to sulphydryl-containing constituents in the cell is in harmony with all the existing data.

The present work thus supports the evidence that such a combination takes place between 1:2:5:6-dibenzanthracene and sulphur-containing substances, but has shown that the cause of its growth-inhibitory action is more subtle and is not a direct result of this reaction.

SUMMARY.

Administration of 1:2:5:6-dibenzanthracene to rats maintained on a 5 per cent protein diet produces fatty infiltration of the liver. Protection is afforded by methionine and to some extent by cystine.

This supports the idea that combination with sulphydryl-containing substances is an early stage in the metabolism of carcinogenic substances, but it has been shown that the growth-inhibiting action of 1:2:5:6-dibenzanthracene is not caused by deprivation of the animal of essential sulphur-containing amino acids as a direct result of this reaction. There is no direct relation between sulphur metabolism and growth inhibition.

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THE RELATION BETWEEN GROWTH INHIBITION, TOXICITY AND PROTEIN METABOLISM IN RATS TREATED WITH 1:2:5:6-DIBENZANTHRACENE.

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THE conclusion was reached by Elson, Goulden and Warren (1947) that the growth inhibition produced by 1:2:5:6-dibenzanthracene cannot be explained by the mere deprivation of essential sulphur-containing amino acids, and a search for the true mechanism of this effect has therefore been carried out.

One obvious possibility is the inhibition of the anterior lobe of the hypophysis with consequent suppression of growth hormone secretion. Some experiments to test this idea have been carried out by Lees (1938), who found that control and 1:2:5:6-dibenzanthracene treated rats showed identical responses to administration of anterior pituitary growth hormone extract, and concluded that the mechanism of growth inhibition was not that of inhibition of the pituitary. His experiments, however, do not appear to be very conclusive, since he administered the dibenzanthracene (10 mg.) to his animals when they were 8 weeks old and the pituitary extract only after the animals had attained an age of 23 weeks, at which time the growth-inhibiting effect of the hydrocarbon was no longer apparent, since his treated animals were then showing a slightly higher rate of growth than the controls.

We therefore attempted to determine the best conditions for testing the effect of anterior pituitary growth-promoting extract, and in our first experiments failed to obtain the immediate, constant and long-continued inhibition of growth described by Haddow, Scott and Scott (1937). In seeking the reason for this discrepancy it was noticed that the control animals used by these authors had a growth rate of 1 to 2 g. per rat per day, whereas our animals were being maintained on a diet which resulted in a growth rate of 3–4 g. per rat per day. When our animals were placed on a diet giving a growth rate of 1 to 2 g. per rat per day, on injection of 1:2:5:6-dibenzanthracene we were able to reproduce the immediate growth inhibition described by Haddow, Scott and Scott. An investigation of the rate of growth and metabolism of the animals as controlled by diet, on the growth inhibition of 1:2:5:6-dibenzanthracene and the effect of anterior pituitary growth hormone on this inhibition has been carried out.

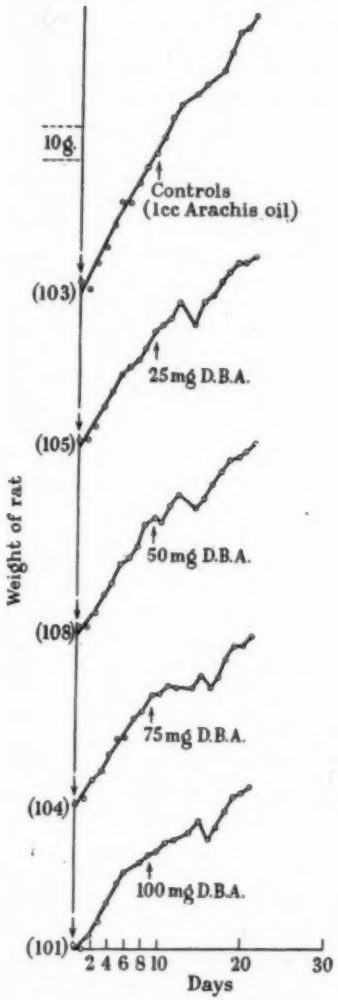

Fig. 1.

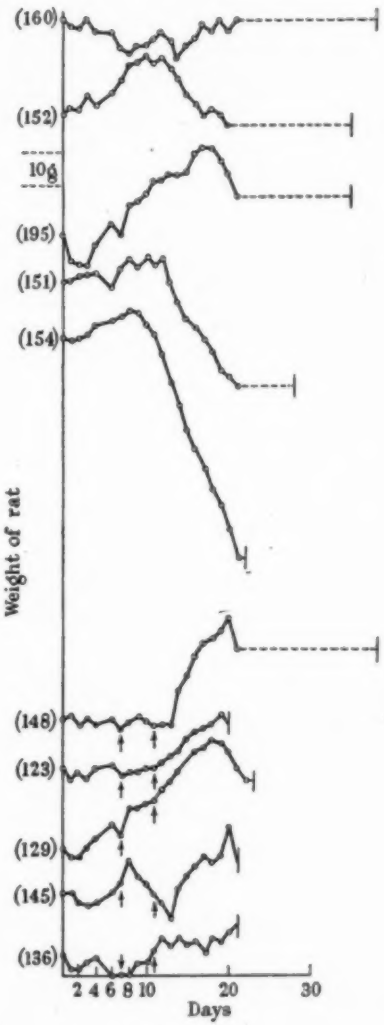

Fig 2.

Fig. 1.—Average growth rates of groups of 10 rats after treatment with 1:2:5:6-dibenzanthracene (i.p. in 1 c.c. arachis oil, given as shown by arrows). The initial average weight (g.) of the group is given in parentheses.

Fig. 2.—Growth rates of individual rats of the group which had received two intraperitoneal injections of 100 mg. 1:2:5:6-dibenzanthracene per rat (Fig. 1). 1 c.c. of anterior pituitary growth hormone was given s.c. daily after the period indicated by the arrows. The initial weight (g.) of each animal is given in parentheses.

EXPERIMENTAL.

In order to find the best conditions for testing the effect of anterior pituitary growth-promoting extract on the growth inhibition produced by 1:2:5:6-dibenzanthracene, groups of 10 rats were given 25 mg., 50 mg., 75 mg. and 100 mg. of the hydrocarbon suspended in arachis oil, by intraperitoneal injection, and the rats weighed daily as a group. In no case, however, even with 100 mg. 1:2:5:6-dibenzanthracene, was any immediate inhibition of the average growth rate of any group of rats observed. After 9 days a second injection of the same amounts of 1:2:5:6-dibenzanthracene was given and again no constant effect was observed, but after several days considerable fluctuations in the growth rates occurred, and eventually 21 days after the first injection all the groups showed a lower average weight than the control group, which had received injections of arachis oil alone. Although the average weight of the group which had received the two injections of 100 mg. 1:2:5:6-dibenzanthracene was slightly lower than that of the other group, there was no direct relation to the amount of hydrocarbon given, and the rats receiving 50 mg. had a higher average weight than the group which had been given 25 mg. (Fig. 1). The rats of the group which had received 100 mg. 1:2:5:6-dibenzanthracene were then transferred to separate cages and the individual weights recorded daily. Half of the animals were then given 0·5 c.c., increased after 4 days to 1 c.c., of anterior pituitary extract (supplied by Professor F. G. Young) and the other five left as controls. The results (Fig. 2) showed that 0·5 c.c. of pituitary extract daily had practically no effect on the growth of the animals, but when the amount was increased to 1 c.c. daily nearly all the rats showed an increase in growth rate, although in four out of five of them this increased growth was followed by their early death. The control group, which had not received the pituitary extract, survived for a significantly longer period, but all lost weight or remained practically stationary during this time. Since 1 c.c. of the pituitary extract daily had been shown by Professor Young to be completely non-toxic, the most probable explanation of this result is that the pituitary extract causes an increase in the general metabolism of the animal, with a consequent increase in the amount of 1:2:5:6-dibenzanthracene being metabolized and increased formation of a toxic metabolic product. An investigation of the growth-inhibiting action of 1:2:5:6-dibenzanthracene under different metabolic conditions was therefore indicated.

The effect of the protein content of the diet on the growth inhibition produced by 1:2:5:6-*dibenzanthracene.*

The failure to reproduce the immediate, constant and long-continued inhibition of growth described by Haddow, Scott and Scott was first fully investigated, weighings being carried out on individual rats kept in separate cages. The rats were maintained on a diet containing 20 per cent protein. The composition of this diet is given in Table I, and the normal daily weight increases of male rats fed on it are 3–4 g.

After intraperitoneal injection of 50 mg. 1:2:5:6-dibenzanthracene in 1 c.c. of arachis oil per rat no immediate decrease in growth rate was observed, and most of the animals continued to grow at approximately the same rate as before the injection for at least 10 days, after which time some fluctuations

occurred, and the majority of the animals began to lose weight fairly steadily after varying periods of time and continued to lose weight until death occurred (Fig. 3).

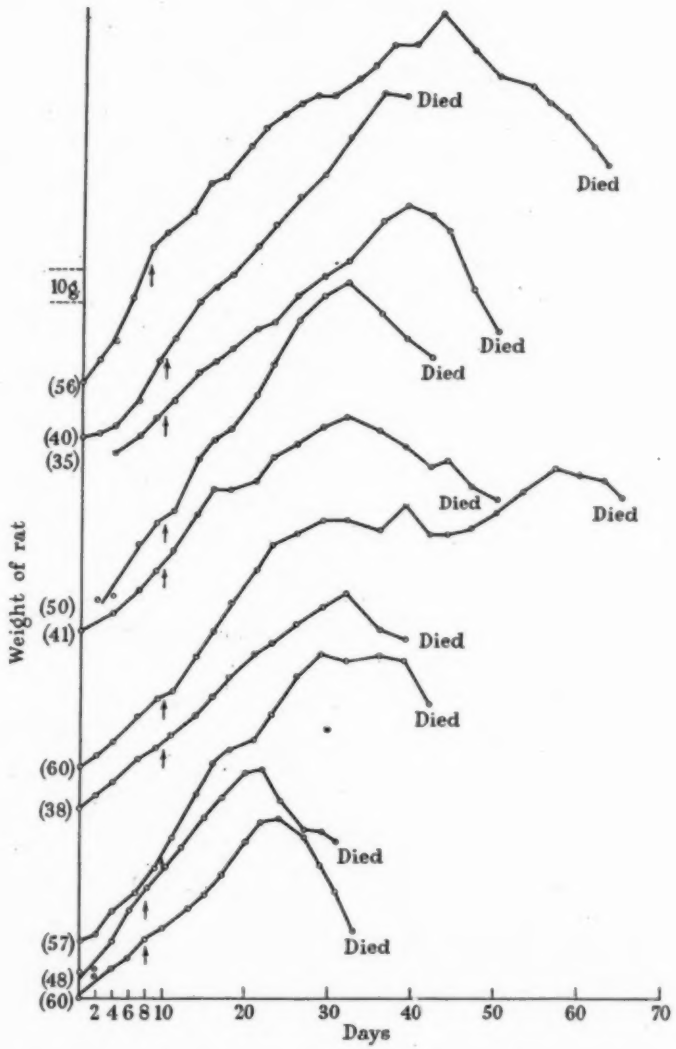

FIG. 3.—Growth rates of individual rats maintained on a high (20 per cent) protein diet before and after treatment (shown by arrow) with 1:2:5:6-dibenzanthracene (50 mg. in 1 c.c. arachis oil per rat i.p.). Figures in parentheses give the initial weight (g.) of the rat.

These results did not support the conclusions of Haddow, Scott and Scott that carcinogenic hydrocarbons produce immediate and long-continued inhibition of growth, but it was noticed that the average growth rates of the rats used by

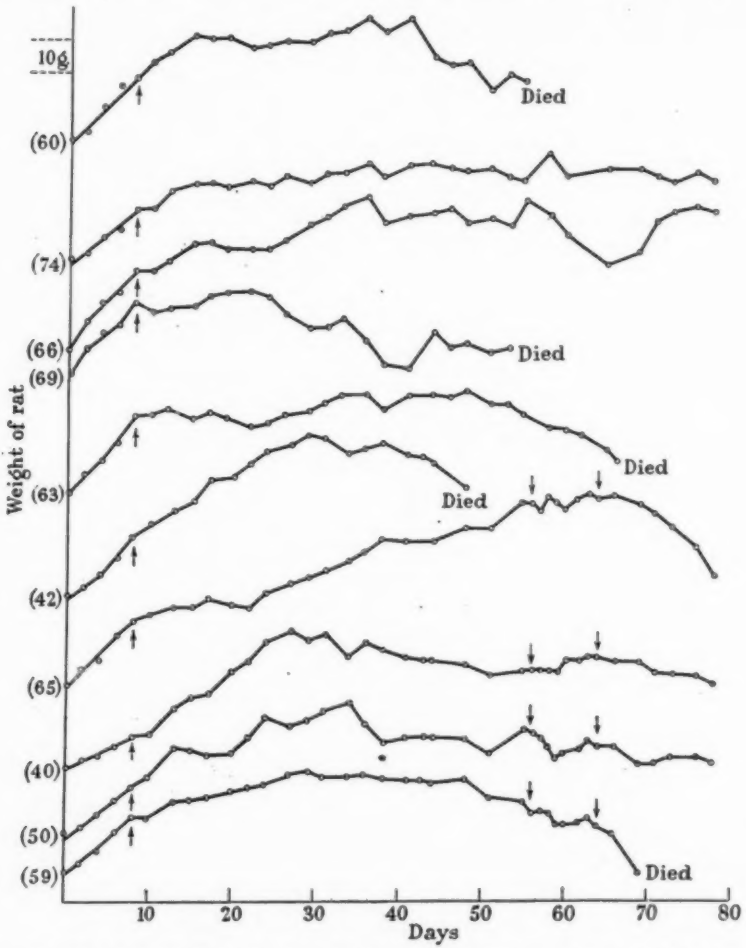

FIG. 4.—Growth rates of individual rats maintained on a low (10 per cent) protein diet before and after treatment (shown by arrow) with 1:2:5:6-dibenzanthracene (50 mg. in 1 c.c. arachis oil per rat i.p.). 1 c.c. of anterior pituitary growth hormone was administered (s.c.) daily during the period shown by the arrows. Figures in parentheses give the initial weight (g.) of the rat.

them were 1·5–2 g. per day, whereas the rats used in the present investigation were growing at the rate of 3–4 g. per day. The experiment was therefore repeated with rats maintained on a 10 per cent protein diet (Table I), on

which their average growth rate was about 2 g. per day. On this diet, after injection of 50 mg. 1:2:5:6-dibenzanthracene, most of the animals showed an immediate inhibition of growth (Fig. 4). This inhibition was prolonged until the death of the animal; in some cases almost complete cessation of growth

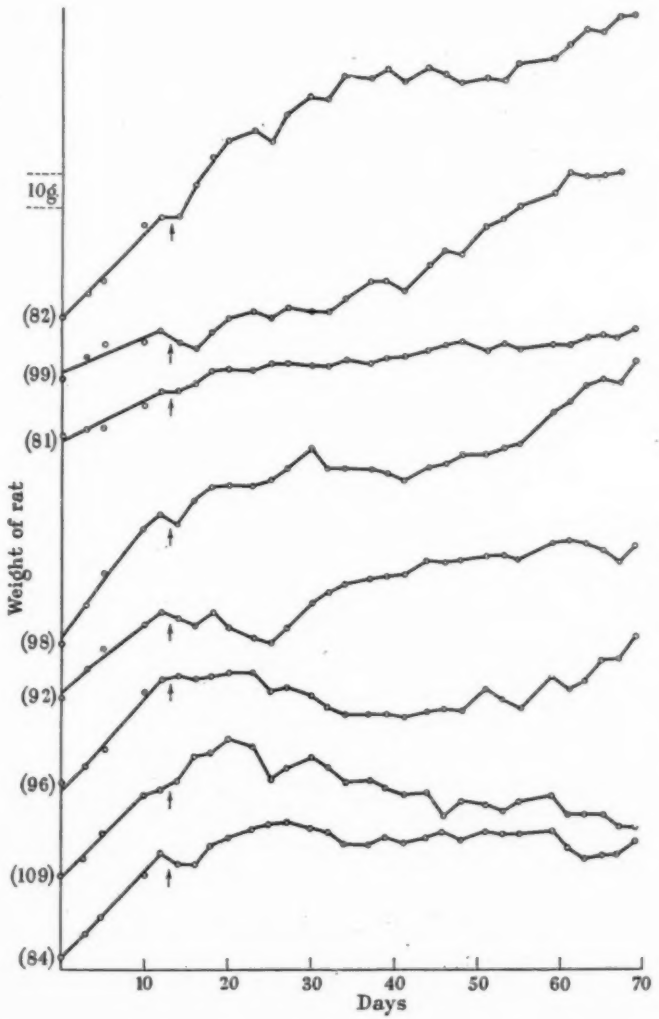

FIG. 5.—Growth rates of individual rats maintained on a 10 per cent protein diet supplemented with 0·4 per cent cystine before and after treatment (shown by arrows) with 1:2:5:6-dibenzanthracene (50 mg. in 1 c.c. arachis oil per rat i.p.). Figures in parentheses give the initial weight (g.) of the rat.

occurred, and the animals maintained a practically constant weight for periods of up to 160 days after the injection of 1:2:5:6-dibenzanthracene.

TABLE I.

20 per cent Protein Diet.		10 per cent Protein Diet.	
National Wheatmeal Flour (85 per cent extraction)	72 per cent	Starch (maize)	80 per cent
Casein	11 ,,	Casein	10 ,,
Milk Powder (full cream)	8 ,,	Bemax	2·5 ,,
Bemax (Vitamins Ltd.)	2·5 ,,	Margarine	5 ,,
Margarine	3 ,,	$CaCo_3$	0·5 ,,
$CaCo_3$	1 ,,	Salt Mixture	1 ,,
Salt Mixture (Glaxo Laboratories Ltd.)	1 ,,	Cod Liver Oil	1 ,,
Cod Liver Oil	1·5 ,,		

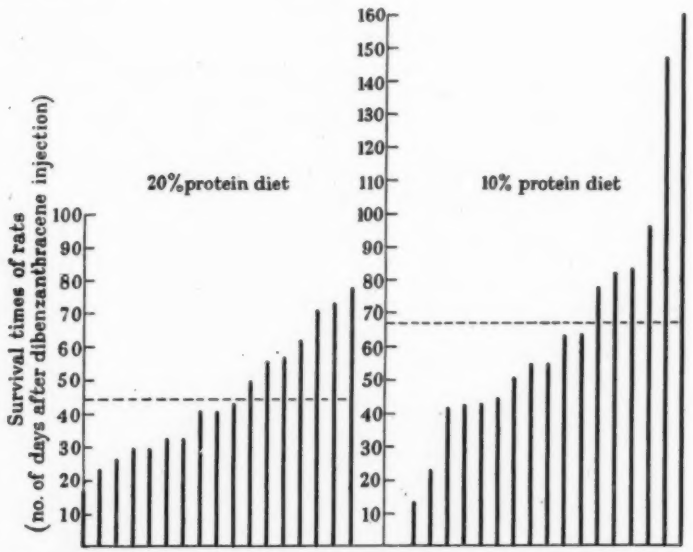

FIG. 6.—Survival times of the rats maintained on 20 per cent and on 10 per cent protein diets after treatment with 1:2:5:6-dibenzanthracene (50 mg. in 1 c.c. arachis oil per rat i.p.).

Administration of anterior pituitary extract to four of these animals, whose growth had been almost completely arrested, failed to bring about any increased growth comparable with that which resulted with the animals on the 20 per cent protein diet, nor did it in this case appear to produce any increased toxic effect of the hydrocarbon.

A further proof that this inhibition of growth is not the result of deprivation of the animals of sulphur-containing amino acids was obtained in an experiment in which the 10 per cent. protein diet was supplemented with an amount of

cystine approximately equal to that which would be obtainable by the animal on the 20 per cent protein diet. The immediate growth inhibition which resulted

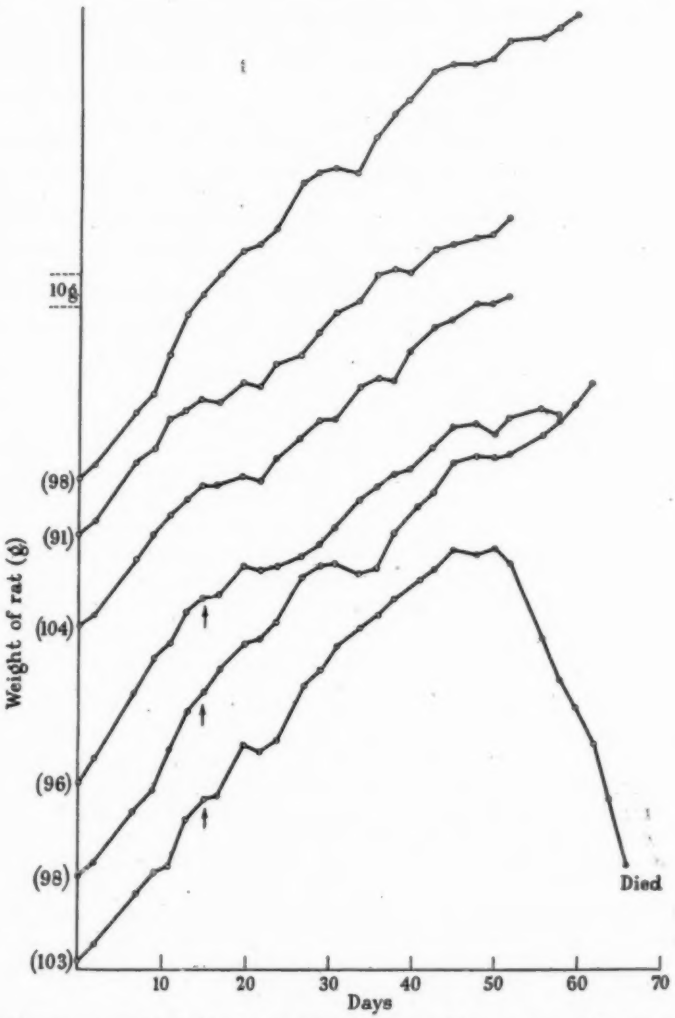

FIG. 7.—Growth rates of individual rats maintained on a 25 per cent protein, 65 per cent starch diet and of rats on the same diet treated (shown by arrow) with 1:2:5:6-dibenzanthracene (50 mg. in 1 c.c. arachis oil per rat). Figures in parentheses give the initial weight (g.) of the rat.

on injection of 1:2:5:6-dibenzanthracene to the animals on this cystine-supplemented diet was scarcely distinguishable from that obtained with the

unsupplemented 10 per cent protein diet, the addition of cystine having entirely failed to reproduce the behaviour shown by the rats on the 20 per cent protein diet (Fig. 5).

In order to ascertain as far as possible that the difference in growth inhibition shown by 1:2:5:6-dibenzanthracene on high and low protein diets is due solely to the protein content and not to any other constituent, an experiment was carried out with a 25 per cent protein diet, in which all the protein was present as casein, and which differed from the 10 per cent protein diet only in that 15 per cent of the starch was replaced by casein. The initial weights of the animals used in this experiment were rather higher than previously (about 100 g.), and since the growth curve for animals much above this weight begins to deviate from the practically straight line curve given by younger animals, growth curves for control animals throughout the period of the experiment were obtained for comparison. The curves are given in Fig. 7. No significant difference in growth rate between the 1:2:5:6-dibenzanthracene-treated and the control animals was observed for about the first 20 days after injection, but the treated animals then began to show considerable fluctuations in weight, and one animal lost weight very rapidly until death ensued. The behaviour was entirely comparable with that of the animals on the 20 per cent protein diet (Fig. 3). Comparison of this result with the immediate growth inhibition produced by 1:2:5:6-dibenzanthracene in animals maintained on the exactly similar 10 per cent protein diet shows that the increased protein content of the diet is the protective factor.

The toxicity of 1:2:5:6-*dibenzanthracene to rats maintained on high and low protein diets.*

Although administration of 50 mg. 1:2:5:6-dibenzanthracene to rats maintained on a 20 per cent protein diet causes no immediate inhibition of growth, a delayed toxic effect occurs which ultimately results in death of all the animals. When the length of time survival of the dibenzanthracene-treated rats on the 20 per cent protein diet is compared with that of the similarly treated animals maintained on the 10 per cent protein diet, as shown in Fig. 6, it is seen that although in the latter group there is an immediate growth inhibitory action, the animals survive for a longer period after treatment. (Mean survival time of 17 animals on the 20 per cent protein diet was 44 days after dibenzanthracene injection, whilst that of the same number of animals on the 10 per cent protein diet was 67 days.)

Effect of 1:2:5:6-*dibenzanthracene on tail growth in rats maintained on a* 20 *per cent protein diet.*

An attempt was made to see if any growth-inhibiting action of 1:2:5:6-dibenzanthracene could be demonstrated by measuring the increase in tail length of rats treated with this hydrocarbon.

Young male rats of about 50 g. body weight were divided into 3 groups of approximately the same average body weight. The animals were lightly anaesthetized with ether, placed on X-ray film in the prone position, and skiagrams taken of the bones from the pelvic region to the tip of the tail. On the same day the rats were injected. Group I received 1 ml. of arachis oil intraperitoneally; Group II, 25 mg. dibenzanthracene suspended in 1 ml. oil; Group

III, 50 mg. dibenzanthracene suspended in oil. Skiagrams of the tails of the animals in all three groups were taken on the 7th, 14th and 21st day after the injection.

At the end of 21 days there was no significant difference in tail growth between animals of the three groups. Measurement of the skiagrams showed that the tails of group I (control group) had increased in length by 44 mm., those of Group II by 42 mm., while in Group III the elongation was 41 mm. These figures are the average increases in tail length for the groups of five animals.

The technique described is based on that given by Gaarenstrom and Levie (1939). These authors found that the synthetic oestrogen stilboestrol had a pronounced inhibitory action on the growth of the rat's tail. In order to compare the action of this substance with that of dibenzanthracene, 10 rats were injected daily with 0·25 mg. of stilboestrol and the tail lengths measured skiagrammatically on the 1st, 7th, 14th and 21st day. A total of 5 mg. of stilboestrol was injected into each rat. In all but two of the animals growth of the tail was markedly inhibited, and in 3 rats tail growth practically ceased. The average increase in tail length for the group of 10 rats was 10 mm. in 21 days—a figure significantly less than that found in either of the dibenzanthracene injected groups.

DISCUSSION.

In considering the nature of the growth-inhibiting action of 1:2:5:6-dibenzanthracene, the most remarkable finding is the influence of diet on the growth-inhibitory action. The difference in the diets used is mainly of the protein content and, since the fat content of the diets is the same, their total calorific values do not differ appreciably. It would thus appear that the protective action of the 20 per cent protein diet is directly attributable to its high protein content. This is confirmed by the protective action of the 25 per cent protein diet, which differs from the unprotective 10 per cent protein diet only in protein content.

The growth-inhibitory action of the hydrocarbon does not appear to be the result of a direct action on the anterior lobe of the pituitary gland causing interference with growth hormone secretion. Administration of growth hormone to rats maintained on the high protein diet and treated with dibenzanthracene does, in most cases, bring about some increase in growth, but appears at the same time to increase the toxic action of the hydrocarbon since this growth increase is rapidly followed by the death of the animal. Dibenzanthracene is only very slightly soluble in body fluids, and when administered by intraperitoneal injection remains in the peritoneum for a considerable period of time. Metabolism of the compound probably takes place by combination with sulphydryl-containing substances as its first stage. Since this combination is not the direct cause of its growth-inhibiting action (Elson, Goulden and Warren, 1947), it is probable that a later stage metabolic product is responsible for this effect. One of the effects of the growth hormone is to increase the total metabolism of the animal, and it seems likely that this results in increased absorption and metabolism of the dibenzanthracene from the peritoneum of the treated animal. The resulting increased accumulation of the toxic metabolic product causes the death of the animal. On the low protein diet when the growth of the animal has been completely inhibited by dibenzanthracene administration of

growth hormone has no effect. On the 20 per cent protein diet 1:2:5:6-dibenzanthracene has no inhibiting action on tail growth in rats, although a marked inhibitory effect was obtained with stilboestrol. Gaarenstrom and Levie (1939) find a close correlation between the effect of oestrogens on body weight and bone development, and conclude that inhibition of body weight increase by stilboestrol is consequent upon the disturbance of bone growth. Griffiths and Young (1942) showed that the growth-inhibitory action of stilboestrol can be overcome by treatment with a suitable dose of anterior pituitary growth-promoting extract.

The mechanism of the growth-inhibiting action of stilboestrol is thus different from that of 1:2:5:6-dibenzanthracene, and the present results show that it is not related to the protein content of the diet as in the case with the latter compound. Although it inhibits the growth of the animal profoundly, stilboestrol has been found by Professor Haddow to have practically no inhibitory effect on the growth of the Walker carcinoma 256 (personal communication).

The high protein diet only affords temporary protection from the growth-inhibiting action of 1:2:5:6-dibenzanthracene (50 mg./100 g.). After a time (usually at least 14 days) the animal begins to lose weight rapidly until death ensues. It appears that accumulation of toxic metabolic products finally results in a breakdown occurring, although no very obvious pathological changes were observed *post mortem*.

With animals maintained on the low protein diet, although an immediate growth-inhibiting effect results on injection of dibenzanthracene, the survival time of the animals is longer than that of those similarly treated but maintained on the high protein diet. Presumably in these animals the rate of absorption and metabolism of the hydrocarbon is lower than that of the animals on the high protein diet, and a dynamic equilibrium is attained between the rate of production of the toxic metabolic product and the total metabolism of the animal.

The fact that the nature of the growth-inhibitory action of 1:2:5:6-dibenzanthracene is dependent on the protein content of the diet suggests that the growth inhibition produced by carcinogenic compounds of this type is brought about by a direct interference with protein metabolism resulting in the prevention of protein synthesis.

SUMMARY.

A direct relation has been found between the protein content of the diet and the growth-inhibiting action of 1:2:5:6-dibenzanthracene. On a 20 per cent protein diet one intraperitoneal injection of 50 mg. of 1:2:5:6-dibenzanthracene per rat usually results in very little growth inhibition for about 14 days, after which the animals, at varying intervals, lose weight rapidly and die. On a 10 per cent protein diet immediate inhibition of growth occurs after injection of the dibenzanthracene (50 mg./100 g.) and persists for a very prolonged period, usually until the death of the animal, although the average survival time of the treated animals on the 10 per cent protein diet is significantly longer than that of those on the 20 per cent protein diet.

The growth-inhibiting action of 1:2:5:6-dibenzanthracene is different from that of stilboestrol, and is not the result of interference with pituitary growth hormone. It is suggested that it is caused by an interference with the availability or the actual synthesis of protein necessary for cellular growth.

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THE INHIBITORY ACTION OF 1:2:5:6-DIBENZANTHRACENE ON THE GROWTH OF THE WALKER CARCINOMA 256 IN RATS MAINTAINED ON HIGH AND LOW PROTEIN DIETS.

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THE protein content of the diet has been shown to have a profound effect on the inhibition of body growth of rats which is produced by 1:2:5:6-dibenzanthracene (Elson and Warren, 1947). If the tumour inhibiting properties of this hydrocarbon (Haddow, 1935; Haddow and Robinson, 1937) are related to its property of body growth inhibition, it seemed probable that the degree of tumour inhibition obtained with it might also be directly influenced by the protein content of the diet. Experiments to test this have now been carried out.

EXPERIMENTAL.

Inhibition of tumour growth by 1:2:5:6-dibenzanthracene in animals maintained on 20 per cent and 10 per cent protein diets.

One group of 18 male Wistar rats was maintained on a high (20 per cent) protein diet, and another similar group of 18 animals on a low (10 per cent) protein diet. The composition of the diets was the same as those used for the body growth inhibition experiments (Elson and Warren, 1947, Table I). The animals were kept in separate cages and weighed every 2 or 3 days. After being on the diet for 14 days both groups were implanted with the Walker carcinoma 256, and 9 animals of each group were later the same day also given an intraperitoneal injection of 50 mg. 1:2:5:6-dibenzanthracene in 1 c.c. arachis oil, the remaining 9 animals of each group acting as controls and receiving an injection of 1 c.c. arachis oil alone. The weighing of the animals was continued during the development of the tumours, and an index of the size of the tumours obtained after

7 days and again after 10 days by measuring along two axes with calipers. According to these measurements very little difference was observed between the size of the tumours of the control and dibenzanthracene treated animals in the high protein group after 7 days, and the tumours of the treated animals appeared to be only slightly smaller than those of the controls after 10 days.

In the low protein group, however, the measurements on the 7th and on the 10th days showed that there was considerable inhibition of tumour growth in the animals treated with dibenzanthracene, while the tumours of the control animals had grown to about the same size as those of the controls of the high protein group. After 11 days the animals were killed and the tumours dissected out and weighed. The weights are given in Table I. Very little tumour inhibition was obtained when the animals were maintained on the high protein diet, the ratio of the average weight of the tumours of the control animals to the average weight of the tumours of the dibenzanthracene treated animals (C/T) being 1·8, whereas in the animals maintained on the low protein diet a marked inhibition of tumour growth occurred (C/T = 4·0).

TABLE I.—*Action of* 1:2:5:6-*Dibenzanthracene on Walker Rat Carcinoma* 256 *in Animals Maintained on* 20 *per cent and* 10 *per cent Protein Diets.*

50 mg. Dibenzanthracene/100 g. rat. Weight of tumours after 11 days.

High (20 per cent) protein diet.		Low (10 per cent) protein diet.	
Controls.	1:2:5:6-dibenzanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.
10·5 g.	5·5 g.	20·5 g.	3·0 g.
16.0 g.	13·0 g.	10·5 g.	5·0 g.
13·5 g.	7·5 g.	14·0 g.	2·0 g.
17·0 g.	7·5 g.	17·0 g.	6·0 g.
11·0 g.	3·0 g.	17·0 g.	3·0 g.
14·0 g.	11·0 g.	13·0 g.	4·0 g.
18·5 g.	11·0 g.	18·0 g.	2·0 g.
19·0 g.	5·0 g.	14·5 g.	4·5 g.
11·0 g.	7·0 g.	..	5·5 g.
Mean *14·5 g.*	Mean *7·9 g.*	Mean *15·6 g.*	Mean *3· 9g.*
$\frac{C}{T} = 1·8$		$\frac{C}{T} = 4·0$	

By subtracting the weight of the tumour from the weight of the tumour-bearing rat when killed, the average total gain in weight of the animal minus tumour in the 11 days after implantation of the tumour was obtained and is given in Table II. In considering the growth of the rat in relation to the growth

TABLE II.—*Average Total Gain in Weight of Rat Minus Tumour during Period of Tumour Growth.*

High (20 per cent) protein diet.		Low (10 per cent) protein diet.	
Controls.	1:2:5:6-dibenxanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.
4·4 g.	8·6 g.	—6·2 g.	1·8 g.

of the tumour, at first both rat and tumour increase in weight, but after a time the tumour gains weight very rapidly and begins to grow at the expense of the rat. This is seen particularly well in the animals on the 10 per cent protein diet, where after 11 days the control animals have lost an average of 6·2 g., while the tumours in these animals have gained 15·6 g. On the other hand the dibenzanthracene treated animals, in spite of body growth inhibition induced by this compound on the 10 per cent protein diet, gained 1·8 g. in 11 days, while the tumour gained 3·9 g.

Inhibition of tumour growth by 1:2:5:6-*dibenzanthracene in animals maintained on* 20 *per cent,* 10 *per cent and* 5 *per cent protein diets.*

A further tumour inhibition experiment was carried out on animals maintained on 20 per cent, 10 per cent and 5 per cent protein diets. The composition of the 20 per cent and 10 per cent protein diets was the same as that used in the previous experiment, and that of the 5 per cent diet was the same as described in Elson, Goulden and Warren (1947—Table II). 1:2:5:6-Dibenzanthracene (50 mg. per rat in 1 c.c. arachis oil) was administered by intraperitoneal injection to half the animals in each group, in this case 24 hours after they had received the tumour implantation, the control animals receiving at the same time 1 c.c. of arachis oil alone. The tumours were allowed to grow for 13 days after implantation, and the rats then killed and the tumours dissected out and weighed. The weights are given in Table III. Again on the 20 per cent protein diet very

TABLE III.—*Action of* 1:2:5:6-*Dibenzanthracene on Walker Rat Carcinoma* 256 *in Animals Maintained on* 20 *per cent,* 10 *per cent and* 5 *per cent Protein Diets.*

50 mg. Dibenzanthracene/100 g. rat. Weight of tumours after 13 days.

20 per cent protein diet.		10 per cent protein diet.		5 per cent protein diet.	
Controls.	1:2:5:6-dibenzanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.
22·5	16·1 .	14·1	11·0 .	16·6	1·6
23·2	9·5 .	12·6	3·0 .	22·9	6·8
18·4	8·6 .	27·4	14·4 .	6·0	3·4
9·0	6·2 .	24·4	9·4 .	14·4	5·6
18·2	8·3 .	27·1	14·7 .	21·1	2·3
15·6	8·6 .	20·5	6·3 .	13·3	5·8
11·3	13·0 .	24·7	5·0 .	23·0	7·5
8·9	2·7 .	13·0	6·0 .	17·8	1·8
20·0	.. .	20·0	7·8 .	9·3	4·5
5·3	.. .	..	7·2 .	15·8	2·0
Mean 15·2	9·2	Mean 20·4	8·4	Mean 16·0	4·1
	$\frac{C}{T} = 1{\cdot}6$		$\frac{C}{T} = 2{\cdot}5$		$\frac{C}{T} = 4{\cdot}0$

little tumour inhibition was obtained with dibenzanthracene (C/T = 1·6), definite tumour inhibition was obtained on the 10 per cent protein diet (C/T = 2·5), and marked tumour inhibition on the 5 per cent protein diet (C/T = 4·0). The

average total gain in weight of the animals minus tumours in the 13 days after implantation of the tumours is given in Table IV.

TABLE IV.—*Average Total Gain in Weight of Rat Minus Tumour during Period of Tumour Growth.*

20 per cent protein diet.		10 per cent protein diet.		5 per cent protein diet.	
Controls.	1:2:5:6-dibenzanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.	Controls.	1:2:5:6-dibenzanthracene treated.
7·9 g.	12·6 g.	—13·9 g.	4·5 g.	—13·6 g.	—0·1 g.

DISCUSSION.

The mechanism of the tumour inhibitory action of 1:2:5:6-dibenzanthracene appears to be essentially the same as that of its body growth inhibition. Both are influenced to a very large extent by the protein content of the diet, and it is suggested that the inhibition is caused by an interference with the availability, or with the actual synthesis, of protein necessary for cellular growth.

With a dose of 50 mg./100 g. of 1:2:5:6-dibenzanthracene complete growth inhibition for a long period can be obtained in animals maintained on 10 per cent and 5 per cent protein diets. It is not, however, possible to inhibit the growth of the Walker carcinoma 256 completely in these animals, although a very considerable slowing down of the growth rate of the tumour can be obtained. In the early stages of tumour growth both animal and tumour increase in weight, but the tumour gains rapidly and eventually the body weight decreases while the tumour continues to gain. The inhibitor, by slowing the growth of the animal and of the tumour, extends the time required for the latter to reach such proportions that its growth at the expense of the body tissues becomes a major factor. The effect is thus most marked during the early growth of the tumour.

It is highly probable, therefore, that complete control of tumour growth is not attainable with an inhibitor of this type, and could only be achieved by a substance having a more specific action on tumour cells. There is no evidence that 1:2:5:6-dibenzanthracene has any such specific action.

A preliminary examination of 2′-chloro-4-dimethylaminostilbene, one of a series of very powerful tumour inhibiting compounds discovered by Haddow, Harris and Kon (1945), reveals that its action is also almost entirely dependent on the protein content of the diet. On a 20 per cent protein diet no inhibiting effect of the compound on the growth of the Walker carcinoma 256 was observed $\left(\begin{matrix}\text{wt. of tumours of control animals C} \\ \text{wt. of tumours of treated animals T}\end{matrix}\right) = 1{\cdot}1$) whereas on a 5 per cent protein diet a very remarkable inhibition of tumour growth was obtained $\left(\frac{C}{T} = 19{\cdot}0\right)$, although the tumours grew equally well in the control animals maintained on the 5 per cent or the 20 per cent protein diet. Moreover, while 1:2:5:6-dibenzanthracene appears to have a relatively greater inhibiting effect on the growth of the animal than on the growth of the tumour, the reverse is the case with the stilbene derivative. Preliminary evidence thus indicates that the growth inhibition shown by this latter type of compound may be associated with a more specific effect on the synthesis of proteins required by the tumour cell.

Some tentative idea of the nature of the inhibiting action of carcinogens such as 1:2:5:6-dibenzanthracene on the growth of cells may be deduced from the present result considered in conjunction with work on the inhibition of enzyme systems by metabolic products of carcinogenic azo compounds, and on the metabolism of aromatic amines. It is very probable that aromatic compounds of this type combine with sulphydryl-containing substances at an early stage in their metabolism. This is then followed by oxidation, and the substance is normally finally excreted as an ethereal sulphate. In the case of the carcinogenic and tumour inhibiting amine 4-aminostilbene it has been found that this elimination mechanism apparently breaks down at some stage, and instead of the compound being eliminated as an ethereal sulphate, free 4-amino-4′-hydroxystilbene is formed, part of which is then excreted conjugated with glucuronic acid (Elson, Goulden and Warren, 1946). Free amino hydroxy compounds of this type are capable of being oxidized *in vivo* by enzymes such as cytochrome oxidase to quinonoid derivatives which are powerful inhibitors of a large number of enzyme systems, particularly those dependent on free sulphydryl groups for their activity (Elson and Hoch-Ligeti, 1946; Elson, 1947). The formation from 1:2:5:6-dibenzanthracene of similar products toxic to enzymes may well be a factor in its interference with processes of protein metabolism and synthesis.

Since, according to the work of Caspersson and others (Caspersson and Santesson, 1942), protein synthesis is associated with high concentrations of nucleic acids in the cell, and the nucleic acid concentration is intimately connected with cell division, the probable inhibition of protein synthesis by 1:2:5:6-dibenzanthracene suggests consequences in the disturbance of nucleoprotein metabolism which may be directly connected with the process of carcinogenesis.

SUMMARY.

The inhibiting action of 1:2:5:6-dibenzanthracene on the growth of the Walker rat carcinoma 256 has been found to be dependent on the protein content of the diet. With animals maintained on a 20 per cent protein diet very little growth inhibitory action is observed, on a 10 per cent protein diet moderate inhibition, and on a 5 per cent protein diet marked inhibition of tumour growth can be obtained.

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